I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated below with Express Mail Label No. EV 775249569 US and is addressed to: Mail Stop Hatch-Waxman PTE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on

20 November 2009
(Date of Deposit)

Deborah Pishock
(Name of person mailing paper)

(Signature)

20 November 2009
(Date of Signature)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No.: 6,902,734 Issued: June 7, 2005
Expiration Date: July 27, 2022

Inventors: Jill Giles-Komar; David M. Knight; David Peritt; Bernard Scallon; David Shealy
Title: ANTI-IL-12 ANTIBODIES AND COMPOSITIONS THEREOF

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPLICATION FOR EXTENSION OF PATENT TERM (37 C.F.R. § 1.740)

Pursuant to 35 U.S.C. § 156 and 37 C.F.R. § 1.740, Centocor Ortho Biotech Inc. ("Applicant") as Assignee and patent owner of the above-captioned patent, hereby petitions for extension of U.S. Patent No. 6,902,734 (the '734 Patent). As permitted by 37 C.F.R. § 1.785(b) and MPEP § 2761, Applicant is concurrently filing a request for patent term extension of U.S. Patent No. 7,166,285 based upon the same regulatory review period.

In support of such Petition, Applicant provides the following information:

I. SIGNATURE REQUIREMENTS (37 C.F.R. §1.730)

A. IDENTIFICATION OF PERSON(S) SUBMITTING THE APPLICATION

I, Eric A. Dichter, represent that I am a registered practitioner appointed by the patent owner of record. A Power of Attorney, authorizing Eric A. Dichter to act on behalf of the Patent Owner is attached hereto as **Exhibit 6**.

B. RECORDAL OF ASSIGNMENT IN PTO

The application for the '734 Patent, U.S. Serial No. 09/920,262, filed August 1, 2001, claiming priority from U.S. Provisional Application No. 60/236,827, filed September 29, 2000, and U.S. Provisional Application No. 60/223,358, was filed August 7, 2000. An assignment of U.S. Serial No. 09/920,262 was recorded: Date: August 31, 2001 at Reel/Frame: 012151/0217 from the named inventors to Centocor, Inc. The successor entity to Centocor, Inc. is Centocor Ortho Biotech Inc. A merger document changing the entity owning U.S. Patent No. 6,902,734 from Centocor, Inc. to Centocor Ortho Biotech Inc. was recorded: Date: November 13, 2009 at Reel/Frame: 023510/0420.

II. APPLICATION REQUIREMENTS (37 C.F.R. §1.740)

A. IDENTIFICATION OF APPROVED PRODUCT (1.740(a)(1))

The United States Food and Drug Administration ("FDA") has approved Biologics License Application ("BLA") No. 125261/0 for STELARA™ (ustekinumab). The active ingredient of STELARA™ is ustekinumab. A copy of the FDA Approval Letter and the approved labeling is attached hereto as **Exhibit 1**.

The active ingredient in the approved product, ustekinumab, is comprised of 1,326 amino acids. The amino acid sequence for ustekinumab is as follows:

(A)	
FW1	EVQLVQSGAEVKKPGESLKISCKGSGYSFT
CDR1	TYWLG
FW2	WVRQMPGKGLDWIG
CDR2	IMSPVDSDIRYSPSFQG
FW3	QVTMSVDKSITTAYLQWNSLKASDTAMYYCAR
CDR3	RRPGQGYFDF
FW4	WGQGTLVTVSS
CH1	SSTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
	HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
HINGE	EPKSCDKTHTCPPCPAPELLG
CH2	GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
	AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
	SKAK
CH3	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
	YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS
	LSLSPGK
(B)	
FW1	DIQMTQSPSSLSASVGDRVTITC
CDR1	RASQGISSWLA
FW2	WYQQKPEKAPKSLIY
CDR2	AASSLQS
FW3	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
CDR3	QQYNIYPYT
FW4	FGQGTKLEIK
CK	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG
	NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK
	SFNRGEC

B. IDENTIFICATION OF THE FEDERAL STATUTE UNDER WHICH REGULATORY REVIEW OCCURRED (1.740(a)(2))

Regulatory review for this product occurred under Section 351(a) of the Public Health Service Act (42 U.S.C. § 262(a)) (21 C.F.R. §§ 314 and 601) and Section 505(i) of the Federal Food Drug & Cosmetic Act ("FDC Act") 21 U.S.C. § 355(i) (21 C.F.R. § 312) (new drugs).

C. DATE OF APPROVAL (1.740(a)(3))

The FDA approved No. 125261/0 for STELARA™ for commercial marketing or use under Section 351(a) of the Public Health Service Act (42 U.S.C. § 262(a)) on September 25, 2009.

D. IDENTIFICATION OF ACTIVE INGREDIENTS AND PREVIOUS APPROVAL INFORMATION (1.740(a)(4))

STELARA™ (ustekinumab) is a human drug product, the sole active ingredient of which is ustekinumab. Ustekinumab has not been previously approved, alone or in combination, for commercial marketing or use under the Federal Food, Drug & Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act.

E. TIMELY SUBMISSION OF APPLICATION (60 DAYS) (1.740(a)(5))

This application is being submitted within the sixty-day time period permitted for submission pursuant to 37 C.F.R. § 1.720(f). The last date this application may be submitted is November 24, 2009.

F. IDENTIFICATION OF PATENT (1.740(a)(6), (7), (8))

Name of the Inventors:	Jill Giles-Komar; David M. Knight; David Peritt; Bernard Scallon; David Shealy
Patent No.	6,902,734
Date of Issue:	June 7, 2005
Date of Original Expiration:	July 27, 2022 (including Patent Term Adjustment under 35 U.S.C. § 154(b))

A copy of the patent, including the entire specification (including claims) and drawings is attached as **Exhibit 2**.

A copy of the U.S. Patent & Trademark Office Maintenance Fee Statements is attached as **Exhibit 3**.

No terminal disclaimer or reexamination certificate has been filed or issued in the '734 patent.

G. IDENTIFICATION OF CLAIMS READING ON THE APPROVED PRODUCT (1.740(a)(9))

The '734 patent claims the active ingredient and a composition of the approved product, which is ustekinumab. The claims directed to the approved product are claims 1-4. A complete claim chart that lists each applicable claim of the '734 patent and demonstrates the manner in which each applicable claim reads on the approved product is attached as **Exhibit 4**.

H. RELEVANT DATES AND INFORMATION (1.740(a)(10))

The '734 Patent claims a human drug.

The effective date of the investigational new drug (IND) application was December 28, 2000, and the IND No. is BB-IND 9590.

The biologics license application (BLA) was initially submitted on November 28, 2007 and was received by the FDA on November 29, 2007. The BLA Number is 125261/0.

The BLA was approved on September 25, 2009.

I. DESCRIPTION OF SIGNIFICANT ACTIVITIES OF APPLICANT DURING REGULATORY REVIEW (1.740(a)(11))

Attached as **Exhibit 5** is a "DESCRIPTION OF SIGNIFICANT ACTIVITIES OF APPLICANT DURING REGULATORY REVIEW" that provides a description of the significant activities undertaken by the marketing applicant during the applicable regulatory review period with respect to the approved Product and the significant dates applicable to such activities.

J. STATEMENT THAT PATENT IS ELIGIBLE FOR EXTENSION (1.740(a)(12))

Attached as **Exhibit 7** is a "STATEMENT THAT PATENT IS ELIGIBLE FOR EXTENSION AND LENGTH OF EXTENSION CLAIMED" that states that in the opinion of the applicant the '734 Patent is eligible for the extension and the length of extension claimed, including how the length of extension was determined.

K. ACKNOWLEDGEMENT OF DUTY OF DISCLOSURE (1.740(a)(13))

I, Eric A. Dichter, the person signing below, acknowledge the duty to disclose to the Director of the U.S. Patent and Trademark Office and to the Secretary of Health and Human Services any information that is material to the determination of entitlement to the extension being sought herein.

L. FEE (1.740(a)(14))

The Application fee due is $1,120.00 (37 C.F.R. § 1.740(a)(14) and § 1.20(j)).

Authorization is hereby made to charge the amount of $1,120.00 to Deposit Account No. 10-0750/CEN0248/EAD.

Please also charge any additional fees required by this paper or credit any overpayment to Deposit Account No. 10-0750.

M. CORRESPONDENCE (1.740(a)(15))

Please direct all inquiries and correspondence relating to this application to:

Philip Johnson, Esq.
Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, NJ 08933

Attn: Eric A. Dichter

Phone: (610) 651-7491
Facsimile: (732) 524-2808

N. COPIES (1.740(a)(15)(b)) and MPEP 2753

Four additional copies of this application are attached, making a total of five copies being submitted.

Conclusion

In conclusion, on the basis of the information provided herein, Applicant respectfully asserts that U.S. Patent No. 6,902,734 is entitled to the requested 425 day extension of its term to September 25, 2023.

Prompt action on this application is respectfully requested.

Date: 20 November 2009

/Eric Dichter/

Reg. No.: 41,708
Tel. No.: 610-651-7491
Customer No.: 000027777

Eric A. Dichter, Esq.
Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, NJ 08933
U.S.A.

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

Our STN: BL 125261/0

BLA APPROVAL
September 25, 2009

Centocor Ortho Biotech, Inc.
Attention: Kim Shields-Tuttle
Senior Director, Global Regulatory Affairs, Immunology
200 Great Valley Parkway
Malvern, PA 19355

Dear Ms. Shields-Tuttle:

Please refer to your biologics license application, dated November 28, 2007, received November 29, 2007, submitted under section 351 of the Public Health Service Act for Stelara™ (ustekinumab).

We acknowledge receipt of your submissions dated November 7 and 21 and December 8, 12, 16, and 18, 2008; January 9, March 5, 23, and 27, April 1, 10, and 27, May 1 and 13, June 25 and 26, July 24, August 5, 11, 12, 14, 17, 21 and 27, and September 11 and 23, 2009.

The January 9, 2009 submission constituted a complete response to our December 18, 2008 action letter.

We have approved your biologics license application for Stelara™ (ustekinumab) effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, Stelara™ (ustekinumab) under your existing Department of Health and Human Services U.S. License No. 1821. Stelara™ (ustekinumab) is indicated for the treatment of adult patients with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy.

Under this license, you are approved to manufacture Stelara™ (ustekinumab) drug substance at Centocor Biologics, LLC in St. Louis, Missouri. The final formulated product will be manufactured, filled, labeled, and packaged at Cilag AG, Schaffhausen, Switzerland. You may label your product with the proprietary name Stelara™ and will market it in 45 mg/0.5 mL and 90 mg/1 mL vials.

Stelara™ (ustekinumab) Pre-Filled Syringe, 45 mg/0.5 mL and 90 mg/1 mL is not approved and review will continue under STN BL 125261/1.

The dating period for Stelara™ (ustekinumab) drug product shall be 12 months from the date of manufacture when stored at 2 – 8 °C. The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period for bulk drug substance

shall be 36 months when stored at -40 °C. We have approved the stability protocols in your license application for the purpose of extending the expiration dating period of your drug product and drug substance under 21 CFR 601.12.

You currently are not required to submit samples of future lots of Stelara™ (ustekinumab) to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of ustekinumab, or in the manufacturing facilities.

REQUIRED PEDIATRIC ASSESSMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients, new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication(s) in pediatric patients unless this requirement is waived, deferred, or inapplicable.

We are deferring submission of your pediatric protocol until December 1, 2022 because pediatric studies should be delayed until additional adult safety and efficacy data have been collected. Pediatric studies are deferred pending analyses of a) safety data from adults in PHOENIX 1 (C0743T08), PHOENIX 2 (C0743T09), the PSOLAR registry, and the Nordic Database Initiative (discussed in items 2, 3, 8, and 9) and b) safety data in pediatric subjects exposed to Stelara™ (ustekinumab) *in utero* or postnatally (described in Items 4, 5, and 6). These safety analyses must establish that there are no safety issues that would preclude study of pediatric subjects. Pediatric studies should not be undertaken until there is agreement with the Agency on the design of such studies.

Your deferred pediatric studies required under section 505B(a) of the Federal Food, Drug, and Cosmetic Act are required postmarketing studies. The status of these postmarketing studies must be reported annually according to 21 CFR 601.70 and section 505B(a)(3)(B) of the Federal Food, Drug, and Cosmetic Act. These required studies are listed below:

1. Conduct studies to evaluate the safety and efficacy of ustekinumab in pediatric subjects with plaque psoriasis.

Pediatric Protocol Submission Date: December 1, 2022

Submit final study reports to this BLA. For administrative purposes, all submissions related to these required pediatric postmarketing studies must be clearly designated "**Required Pediatric Assessment.**"

POSTMARKETING REQUIREMENTS UNDER 505(o)

Section 505(o) of the Federal Food, Drug, and Cosmetic Act (FDCA) authorizes FDA to require holders of approved drug and biological product applications to conduct postmarketing studies and clinical trials for certain purposes, if FDA makes certain findings required by the statute (section 505(o)(3)(A)).

We have determined that an analysis of spontaneous postmarketing adverse events reported under subsection 505(k)(1) of the FDCA will not be sufficient to assess the known risk of serious infection; or to identify unexpected serious risks of malignancy, tuberculosis, opportunistic infections, hypersensitivity reactions, autoimmune disease, neurologic or demyelinating disease, cardiovascular, gastrointestinal or hematologic events, adverse pregnancy and fetal outcomes, adverse effects on immune system development, or altered metabolism of co-administered drugs.

Furthermore, the new pharmacovigilance system that FDA is required to establish under section 505(k)(3) of the FDCA has not yet been established and is not sufficient to assess these serious risks.

Therefore, based on appropriate scientific data, FDA has determined that you are required, pursuant to section 505(o)(3) of the FDCA, to conduct the following studies:

2. Enroll 4,000 Stelara™ (ustekinumab)-treated subjects into the Psoriasis Longitudinal Assessment and Registry, (PSOLAR) and follow for 8 years from the time of enrollment. Subjects will be followed for the occurrence of serious infection, tuberculosis, opportunistic infections, malignancy, hypersensitivity reactions, autoimmune disease, neurologic or demyelinating disease, cardiovascular, gastrointestinal or hematologic adverse events,

   Submit this information according to the following timetable:

Final Protocol Submission:	January 15, 2010
Annual Reports:	2011, 2012, 2013, 2014
Interim Summary Report:	2015
Annual Reports:	2016, 2017, 2018, 2019
Study Completion Date:	December 1, 2019
Final Report Submission:	December 1, 2020

3. Provide data analyses from the Nordic Database Initiative regarding the occurrence of serious infection, tuberculosis, opportunistic infections, malignancy, hypersensitivity reactions, autoimmune disease, neurologic or demyelinating disease, cardiovascular, gastrointestinal or hematologic adverse events with exposure to ustekinumab.

   Submit this information according to the following timetable:

Final Protocol Submission:	January 15, 2010
Annual Reports:	2011, 2012, 2013, 2014
Interim Summary Report:	2015
Annual Reports:	2016, 2017, 2018, 2019
Study Completion Date:	December 15, 2019
Final Report Submission:	December 15, 2020

4. Establish a U.S.-based prospective, observational pregnancy exposure registry that compares the pregnancy and fetal outcomes of women exposed to Stelara™ (ustekinumab) during pregnancy to an unexposed control population. Outcomes of the registry should include major and minor congenital anomalies, spontaneous abortions, stillbirths, elective terminations, adverse effects on immune system development, and other serious adverse pregnancy outcomes. These outcomes should be assessed throughout pregnancy. Infant outcomes should be assessed through at least the first year of life.

   Submit this information according to the following timetable:

Final Protocol Submission:	January 15, 2010
Annual Reports:	2011, 2012, 2013
Study Completion Date:	July 15, 2013
Final Report Submission:	July 15, 2014

5. Provide data analyses from the Pregnancy Research Initiative (study C0168T71).

   Submit this information according to the following timetable:

Final Protocol Submission:	January 15, 2010
Annual Reports:	2011, 2012, 2013, 2014
Interim Summary Report:	2015
Annual Reports:	2016, 2017, 2018, 2019, 2020
Study Completion Date:	December 15, 2020
Final Report Submission:	December 15, 2021

6. Conduct a lactation study in women who are breastfeeding while exposed to Stelara™ (ustekinumab). This study may be conducted in a subset of women enrolled in the U.S.-based pregnancy registry (discussed in PMR # 4) who choose to breastfeed their infants, and should assess for the presence of Stelara™ (ustekinumab) in breast milk and potential adverse effects in nursing infants.

   Submit this information according to the following timetable:

Final Protocol Submission:	January 15, 2010
Annual Reports:	2011, 2012, 2013
Study Completion Date:	July 15, 2013
Final Report Submission:	July 15, 2014

7. Conduct an *in vitro* study to assess whether IL-12 and/or IL-23 modulate expression of major CYP enzymes (i.e., CYP 3A4, CYP 1A2, CYP 2C9, CYP 2C19, and CYP 2D6). If, upon review, there is no marked modulation of any of the major CYP enzyme(s) observed, further exploration would not be necessary.

   The timetable you submitted on August 17, 2009 states that you will conduct this study according to the following timetable:

Final Protocol Submission:	February 2010
Study Completion Date:	July 2010
Final Report Submission:	December 2010

Finally, we have determined that only clinical trials (rather than an observational study) will be sufficient to assess the known risk of serious infection; or to identify unexpected serious risks of malignancy, tuberculosis, opportunistic infections, hypersensitivity reactions, autoimmune disease, neurologic or demyelinating disease, cardiovascular, gastrointestinal or hematologic events, or altered metabolism from co-administered drugs.

Therefore, based on appropriate scientific data, FDA has determined that you are required, pursuant to section 505(o)(3) of the FDCA, to conduct the following clinical trials:

8. Complete the treatment and evaluation of subjects enrolled in the ongoing PHOENIX 1 (C0743T08) trial for a total of 5 years from initial enrollment unless a safety signal is identified that indicates the potential risks of such continued long-term treatment outweigh the benefits. Evaluation of subjects should continue through 5 years (even if treatment is not continued for this duration). Subjects will be followed for the occurrence of serious infection, tuberculosis, opportunistic infections, malignancy, hypersensitivity reactions, autoimmune disease, neurologic or demyelinating disease, cardiovascular, gastrointestinal or hematologic adverse events.

   The timetable you submitted on August 5, 2009 states that you will conduct this trial according to the following timetable:

Final Protocol Submission:	September 2005
Trial Completion Date:	May 2011
Final Report Submission:	January 2012

9. Complete the treatment and evaluation of subjects enrolled in the ongoing PHOENIX 2 (C0743T09) trial for a total of 5 years from initial enrollment unless a safety signal is identified that indicates the potential risks of such continued long-term treatment outweigh the benefits. Evaluation of subjects should continue through 5 years (even if treatment is not continued for this duration). Subjects will be followed for the occurrence of serious infection, tuberculosis, opportunistic infections, malignancy, hypersensitivity

reactions, autoimmune disease, neurologic or demyelinating disease, cardiovascular, gastrointestinal or hematologic adverse events.

The timetable you submitted on August 5, 2009 states that you will conduct this trial according to the following timetable:

Final Protocol Submission:	December 2005
Trial Completion Date:	October 2011
Final Report Submission:	June 2012

10. If the results of the *in vitro* study (discussed under PMR #7) are positive (i.e., if there is marked modulation of any of the major CYP enzyme(s)) conduct a clinical trial to determine the potential of ustekinumab to alter CYP substrate metabolism in psoriasis patients (e.g., using a cocktail of relevant CYP probe drugs).

    The timetable you submitted on August 17, 2009 states that you will conduct this trial according to the following timetable:

Final Protocol Submission:	September 2011
Trial Completion Date:	December 2012
Final Report Submission:	September 2013

Submit the protocols to your IND, with a cross-reference letter to this BLA. Submit all final reports to your BLA. Using the following designators to prominently label all submissions, including supplements, relating to these postmarketing study requirements as appropriate:

- **REQUIRED POSTMARKETING PROTOCOL UNDER 505(o)**
- **REQUIRED POSTMARKETING FINAL REPORT UNDER 505(o)**
- **REQUIRED POSTMARKETING CORRESPONDENCE UNDER 505(o)**

Section 505(o)(3)(E)(ii) of the FDCA requires you to report periodically on the status of any study or clinical trial required under this section. This section also requires you to periodically report to FDA on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Section 506B of the FDCA, as well as 21 CFR 601.70 requires you to report annually on the status of any postmarketing commitments or required studies or clinical trials.

FDA will consider the submission of your annual report under section 506B and 21 CFR 601.70 to satisfy the periodic reporting requirement under section 505(o)(3)(E)(ii), provided that you include the elements listed in 505(o) and 21 CFR 601.70. We remind you that to comply with 505(o), your annual report must also include a report on the status of any study or clinical trial otherwise undertaken to investigate a safety issue. Failure to submit an annual report for studies or clinical trials required under 505(o) on the date required will be considered a violation of FDCA section 505(o)(3)(E)(ii) and could result in enforcement action.

POSTMARKETING COMMITMENTS SUBJECT TO REPORTING REQUIREMENTS OF 21 CFR 601.70

We also acknowledge your written commitments as described in your letter dated August 5, 14, and 17, 2009 as outlined below:

11. Provide information on maintenance of response with dosing intervals longer than every 12 weeks among relevant populations (e.g., subjects whose psoriasis is cleared as measured by PGA and PASI or who have minimal psoriasis). This information will be obtained from a study of at least 300 subjects treated with Stelara™ (ustekinumab) for a minimum of one year.

    The study should not be undertaken until there is agreement with the Agency on the design of your study.

Concept Paper Submission:	March 2010
Draft Protocol Submission:	September 2010
Final Protocol Submission:	December 2010
Final Report Submission:	6 months after completion of study

12. Evaluate approaches to improve drug tolerance in the assay method for anti-drug antibodies (ADA). If a suitable method is developed, it will be applied to assess ADA in patient samples banked from the pivotal trials, if available, and on-going clinical trials. Alternatively, documentation will be submitted to the FDA demonstrating, with due diligence, that such an assay could not be feasibly developed.

Final Report Submission:	December 31, 2012

13. Establish quantitative drug product release and stability specifications for the non-reduced cSDS assay when sufficient commercial experience with the assay has been gained. A proposed specification including justification based on supporting data will be submitted as a prior approval supplement.

Final Report Submission:	September 2011

14. Collect drug product release and stability data to reassess and lower the allowable number of sub-visible particles. A proposed specification including justification based on supporting data will be submitted as a CBE-0 supplement.

Final Report Submission:	September 2010

15. Reassess release and shelf-life specifications for the ustekinumab drug substance and drug product within 2 years from the date of this letter and submit in an annual report.

Final Report Submission: Annual Report 2011

16. Conduct end-of-life concurrent validation of Protein A, anion exchange, and cation exchange resins at the manufacturing scale. The studies will include an assessment of yield, chromatographic profile, and impurities where appropriate. Data will be submitted as a CBE-0 supplement.

Final Report Submission: September 2011

17. Perform reduced scale end-of-life viral removal studies for the anion exchange resin. Study conditions will adequately reflect the manufacturing scale process.

Final Report Submission: September 2010

18. Revise the direct product capture pool SDS-PAGE and IEF stability specifications upon review of available stability data. The proposed specifications, including justification based on supporting data, will be submitted as a CBE-0 supplement.

Final Report Submission: September 2010

19. Develop and validate the Microflow Digital Imaging assay and incorporate this assay into the annual stability testing program with appropriately justified specifications. Alternately, documentation can be submitted to FDA demonstrating with due diligence that this assay could not be feasibly developed.

Final Report Submission: September 2011

20. Perform both IEF and cIEF in parallel for future batches as part of the commercial stability program until sufficient data demonstrate that the cIEF is as stability indicating as the IEF. Data will be submitted as a CBE-30 supplement.

Final Report Submission: September 2011

21. Perform an extensive qualification study for multi-use of the glass syringes which are used for pooling of vials for the visible particle assay to ensure continued effectiveness of the cleaning procedure. Data will be provided within one year of the date of this letter in an annual report.

Final Report Submission: Annual Report 2010

22. Continue the root cause investigation to identify the causative factor(s) that led to increased visible particle counts on stability for the clinical and validation drug product batches. The final report will be provided within one year of the date of this letter in an annual report.

Final Report Submission: Annual Report 2010

23. Develop and implement a bioburden test method that uses an increased sample volume for the determination of bioburden in the pre-harvest (at disconnection of the final harvest bag for each Stage 3 batch) and harvest samples. The acceptance criteria for bioburden in-process controls should be consistent with historical data and reported as CFU/volume tested.

    The revised test method and acceptance criteria including justification based on supporting data will be submitted as a prior approval supplement.

    Final Report Submission: September 2011

We request that you submit clinical protocols to your IND, with a cross-reference letter to this BLA. Submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to this BLA. Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing commitments as appropriate:

- **POSTMARKETING COMMITMENT PROTOCOL**
- **POSTMARKETING COMMITMENT – FINAL STUDY REPORT**
- **POSTMARKETING COMMITMENT CORRESPONDENCE**
- **ANNUAL STATUS REPORT OF POSTMARKETING STUDY COMMITMENTS**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e. pending, ongoing, delayed, terminated, or submitted),
- an explanation of the status including, for clinical studies, the patient accrual rate (i.e. number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (http://www.accessdata.fda.gov/scripts/cder/pmc/index.cfm). Please refer to the February 2006 Guidance for Industry: Reports on the Status of Postmarketing Study Commitments - Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM077374.pdf) for further information.

RISK EVALUATION AND MITIGATION STRATEGY REQUIREMENTS

Section 505-1 of the Federal Food, Drug, and Cosmetic Act (FDCA) authorizes FDA to require the submission of a Risk Evaluation and Mitigation Strategy (REMS) if FDA determines that such a strategy is necessary to ensure that the benefits of the drug outweigh the risks (section 505-1(a)).

Your proposed REMS, submitted on September 23, 2009, and amended and appended to this letter, is approved. The REMS consists of a Medication Guide, a communication plan, and a timetable for submission of assessments of the REMS.

The REMS assessment plan should include but is not limited to the following:

1. Evaluations of dermatologists/healthcare providers' understanding and patients' understanding of the risks of Stelara™ (ustekinumab), including evaluations of the following:

    a. Prescribers' understanding of the risks of Stelara™ (ustekinumab), including the risks of serious infection, RPLS, and malignancy and how to select patients who are appropriate for treatment.

    b. Patients' understanding of the risks of Stelara™ (ustekinumab), including the risks of serious infection, RPLS, and malignancy.

2. A report on periodic assessments of the distribution and dispensing of the Medication Guide in accordance with 21 CFR 208.24.

3. A report on failures to adhere to Medication Guide distribution and dispensing requirements, and corrective actions taken to address noncompliance.

4. A summary of all reported serious infections, RPLS, and malignancies with analysis of adverse event reporting by prescriber type (e.g., dermatologist, nurse, internist, oncologist), when available.

5. Based on the information submitted, an assessment and conclusion of whether the REMS is meeting its goals, and whether modifications to the REMS are needed.

The requirements for assessments of an approved REMS under section 505-1(g)(3) include, in section 505-1(g)(3)(B) and (C), information on the status of any postapproval study or clinical trial required under section 505(o) or otherwise undertaken to investigate a safety issue. You can satisfy these requirements in your REMS assessments by referring to relevant information included in the most recent annual report required under section 506B and 21 CFR 314.81(b)(2)(vii) [or 21 CFR 601.70] and including any updates to the status information since the annual report was prepared. Failure to comply with the REMS assessments provisions in 505-1(g) could result in enforcement action.

We remind you that in addition to the assessments submitted according to the timetable included in the approved REMS, you must submit a REMS assessment and may propose a modification to the approved REMS when you submit a supplemental application for a new indication for use as described in Section 505-1(g)(2)(A) of FDCA.

Prominently identify the submission containing the REMS assessments or proposed modifications with the following wording in bold capital letters at the top of the first page of the submission:

BLA 125261 REMS ASSESSMENT

NEW SUPPLEMENT FOR BLA 125261
PROPOSED REMS MODIFICATION
REMS ASSESSMENT

NEW SUPPLEMENT (NEW INDICATION FOR USE) FOR BLA 125261
REMS ASSESSMENT
PROPOSED REMS MODIFICATION (if included)

If you do not submit electronically, please send 5 copies of REMS-related submissions.

ADVERSE EVENT REPORTING

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). In addition, submit any adverse event reports related to malignancy, serious infections (including opportunistic infections and tuberculosis) and serious neurologic events as 15-day reports. Serious events are defined as events leading to death, hospitalization, disability, or reported as life threatening. You should submit postmarketing adverse experience reports to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at http://www.fda.gov/Safety/MedWatch/HowToReport/ucm166910.htm.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with

processing, testing, packing, labeling, storage, holding, and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA 3486 to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Compliance Risk Management and Surveillance, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Biological product deviations sent by courier or overnight mail should be addressed to Food and Drug Administration, Center for Drug Evaluation and Research, Office of Compliance, Division of Compliance Risk Management and Surveillance, 10903 New Hampshire Avenue, Bldg. 51, Room 4206, Silver Spring, MD 20993-0002.

CONTENT OF LABELING

Within 14 days of the date of this letter, submit content of labeling [21 CFR 601.14(b)] in structured product labeling (SPL) format, as described at http://www.fda.gov/oc/datacouncil/spl.html, that is identical in content to the enclosed labeling (text for the package insert and Medication Guide). For administrative purposes, please designate this submission **"Product Correspondence – Final SPL for approved STN BL 125261/0."** In addition, within 21 days of the date of this letter, amend your pending supplements for the Pre-Filled Syringe (STN BL 125261/1) with content of labeling in SPL format to include the changes approved in this BLA.

CARTON AND IMMEDIATE CONTAINER LABELS

Submit final printed carton and container labels that are identical to the enclosed draft labels as soon as they are available but no more than 30 days after they are printed. Please submit these labels electronically according to the guidance for industry titled *Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (October 2005).* Alternatively, you may submit 12 paper copies, with 6 of the copies individually mounted on heavy-weight paper or similar material. For administrative purposes, designate this submission **"Product Correspondence – Final Printed Carton and Container Labels for approved STN BL 125261/0."** Approval of this submission by FDA is not required before the labeling is used.

Marketing the product with labeling that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

PROMOTIONAL MATERIALS

You may request advisory comments on proposed introductory advertising and promotional labeling. To do so, submit, in triplicate, a cover letter requesting advisory comments, the proposed materials in draft or mock-up form with annotated references, and the package insert(s) to:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Drug Marketing, Advertising, and Communications

5901-B Ammendale Road
Beltsville, MD 20705-1266

As required under 21 CFR 314.81(b)(3)(i), you must submit final promotional materials, and the package insert(s), at the time of initial dissemination or publication, accompanied by a Form FDA 2253. For instruction on completing the Form FDA 2253, see page 2 of the Form. For more information about submission of promotional materials to the Division of Drug Marketing, Advertising, and Communications (DDMAC), see www.fda.gov/cder/ddmac.

If you have any questions, call the Regulatory Project Manager, Sue Kang, at 301-796-4216.

Sincerely,

/John Jenkins, M.D./ September 25, 2009
John Jenkins, M.D. on behalf of Julie Beitz, M.D.
Julie Beitz, M.D.
Director
Office of Drug Evaluation III
Center for Drug Evaluation and Research

Enclosures: Package Insert, Medication Guide, Carton and Container Labels
REMS (including Communication Plan)

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use STELARA™ safely and effectively. See full prescribing information for STELARA™.

STELARA™ (ustekinumab)
Injection, for subcutaneous use
Initial U.S. Approval: 2009

----------INDICATIONS AND USAGE----------

STELARA™ is a human interleukin-12 and -23 antagonist indicated for the treatment of adult patients (18 years or older) with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy. (1)

----------DOSAGE AND ADMINISTRATION----------

STELARA™ is administered by subcutaneous injection. (2)

- For patients weighing ≤100 kg (220 lbs), the recommended dose is 45 mg initially and 4 weeks later, followed by 45 mg every 12 weeks. (2.1)
- For patients weighing >100 kg (220 lbs), the recommended dose is 90 mg initially and 4 weeks later, followed by 90 mg every 12 weeks. (2.1)

----------DOSAGE FORMS AND STRENGTHS----------

- 45 mg/0.5 mL in a single-use glass vial (3)
- 90 mg/1 mL in a single-use glass vial (3)

----------CONTRAINDICATIONS----------

None (4)

----------WARNINGS AND PRECAUTIONS----------

- Infections: Serious infections have occurred. Do not start STELARA™ during any clinically important active infection. If a serious infection develops, stop STELARA™ until the infection resolves. (5.1)
- Theoretical Risk for Particular Infections: Serious infections from mycobacteria, salmonella and Bacillus Calmette-Guerin (BCG) vaccinations have been reported in patients genetically deficient in IL-12/IL-23. Diagnostic tests for these infections should be considered as dictated by clinical circumstances. (5.2)
- Tuberculosis (TB) evaluation: Evaluate patients for TB prior to initiating treatment with STELARA™. Initiate treatment of latent TB before administering STELARA™. (5.3)
- Malignancies: STELARA™ may increase risk of malignancy. The safety of STELARA™ in patients with a history of or a known malignancy has not been evaluated. (5.4)
- Reversible Posterior Leukoencephalopathy Syndrome (RPLS): One case was reported. If suspected, treat promptly and discontinue STELARA™. (5.5)

----------ADVERSE REACTIONS----------

Most common adverse reactions (incidence >3% and greater than with placebo): Nasopharyngitis, upper respiratory tract infection, headache, and fatigue. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Centocor Ortho Biotech Inc. at 1-800-457-6399 or FDA at 1-800-FDA-1088 or *www.fda.gov/medwatch.*

----------DRUG INTERACTIONS----------

- Live vaccines: Should not be given with STELARA™. (7.1)
- Concomitant therapy: The safety of concomitant use of STELARA™ with immunosuppressants or phototherapy has not been evaluated. (7.2)

See 17 for PATIENT COUNSELING INFORMATION and MEDICATION GUIDE

09/2009

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

STELARA™ is indicated for the treatment of adult patients (18 years or older) with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy.

2 DOSAGE AND ADMINISTRATION

2.1 Dosing

STELARA™ is administered by subcutaneous injection.

- For patients weighing ≤100 kg (220 lbs), the recommended dose is 45 mg initially and 4 weeks later, followed by 45 mg every 12 weeks.

- For patients weighing >100 kg (220 lbs), the recommended dose is 90 mg initially and 4 weeks later, followed by 90 mg every 12 weeks.

In subjects weighing >100 kg, 45 mg was also shown to be efficacious. However, 90 mg resulted in greater efficacy in these subjects *[see Clinical Studies (14)]*.

The safety and efficacy of STELARA™ have not been evaluated beyond two years.

2.2 General Considerations for Administration

STELARA™ is intended for subcutaneous administration under the supervision of a physician.

Prior to administration, STELARA™ should be visually inspected for particulate matter and discoloration. STELARA™ is colorless to light yellow and may contain a few small translucent or white particles. STELARA™ should not be used if it is discolored or cloudy, or if other particulate matter is present. STELARA™ does not contain preservatives; therefore, any unused product remaining in the vial and/or syringe should be discarded.

It is recommended each injection be administered using a 27 gauge, ½ inch needle at a different anatomic location (such as upper arms, gluteal regions, thighs, or any quadrant of abdomen) than the previous injection, and not into areas where the skin is tender, bruised, erythematous, or indurated.

STELARA™ should only be administered by a healthcare provider. STELARA™ should only be administered to patients who will be closely monitored and have regular follow-up visits with a physician.

3 DOSAGE FORMS AND STRENGTHS

STELARA™ contains 90 mg ustekinumab per mL.

- 45 mg/0.5 mL in a single-use vial
- 90 mg/1 mL in a single-use vial

4 CONTRAINDICATIONS

None.

5 WARNINGS AND PRECAUTIONS

5.1 Infections
STELARA™ may increase the risk of infections and reactivation of latent infections. Serious bacterial, fungal, and viral infections were observed in subjects receiving STELARA™ *[see Adverse Reactions (6.1)]*.

STELARA™ should not be given to patients with any clinically important active infection. STELARA™ should not be administered until the infection resolves or is adequately treated. Instruct patients to seek medical advice if signs or symptoms suggestive of an infection occur. Exercise caution when considering the use of STELARA™ in patients with a chronic infection or a history of recurrent infection.

Serious infections requiring hospitalization occurred in the psoriasis development program. These serious infections included cellulitis, diverticulitis, osteomyelitis, viral infections, gastroenteritis, pneumonia, and urinary tract infections.

5.2 Theoretical Risk for Vulnerability to Particular Infections
Individuals genetically deficient in IL-12/IL-23 are particularly vulnerable to disseminated infections from mycobacteria (including nontuberculous, environmental mycobacteria), salmonella (including nontyphi strains), and Bacillus Calmette-Guerin (BCG) vaccinations. Serious infections and fatal outcomes have been reported in such patients.

It is not known whether patients with pharmacologic blockade of IL-12/IL-23 from treatment with STELARA™ will be susceptible to these types of infections. Appropriate diagnostic testing should be considered, e.g., tissue culture, stool culture, as dictated by clinical circumstances.

5.3 Pre-treatment Evaluation for Tuberculosis
Evaluate patients for tuberculosis infection prior to initiating treatment with STELARA™. Do not administer STELARA™ to patients with active tuberculosis. Initiate treatment of latent tuberculosis prior to administering STELARA™. Consider anti-tuberculosis therapy prior to initiation of STELARA™ in patients with a past history of latent or active tuberculosis in whom an adequate course of treatment cannot be confirmed. Patients receiving STELARA™ should be monitored closely for signs and symptoms of active tuberculosis during and after treatment.

5.4 Malignancies
STELARA™ is an immunosuppressant and may increase the risk of malignancy. Malignancies were reported among subjects who received STELARA™ in clinical studies *[see Adverse Reactions (6.1)]*. In rodent models, inhibition of IL-12/IL-23p40 increased the risk of malignancy *[see Nonclinical Toxicology (13)]*.

The safety of STELARA™ has not been evaluated in patients who have a history of malignancy or who have a known malignancy.

5.5 Reversible Posterior Leukoencephalopathy Syndrome
One case of reversible posterior leukoencephalopathy syndrome (RPLS) was observed during the clinical development program which included 3523 STELARA™-treated subjects. The subject, who had received 12 doses of STELARA™ over approximately two years, presented with headache,

seizures and confusion. No additional STELARA™ injections were administered and the subject fully recovered with appropriate treatment.

RPLS is a neurological disorder, which is not caused by demyelination or a known infectious agent. RPLS can present with headache, seizures, confusion and visual disturbances. Conditions with which it has been associated include preeclampsia, eclampsia, acute hypertension, cytotoxic agents and immunosuppressive therapy. Fatal outcomes have been reported.

If RPLS is suspected, STELARA™ should be discontinued and appropriate treatment administered.

5.6 Immunizations

Prior to initiating therapy with STELARA™, patients should receive all immunizations appropriate for age as recommended by current immunization guidelines. Patients being treated with STELARA™ should not receive live vaccines. BCG vaccines should not be given during treatment with STELARA™ or for one year prior to initiating treatment or one year following discontinuation of treatment. Caution is advised when administering live vaccines to household contacts of patients receiving STELARA™ because of the potential risk for shedding from the household contact and transmission to patient.

Non-live vaccinations received during a course of STELARA™ may not elicit an immune response sufficient to prevent disease.

5.7 Concomitant Therapies

The safety of STELARA™ in combination with other immunosuppressive agents or phototherapy has not been evaluated. Ultraviolet-induced skin cancers developed earlier and more frequently in mice genetically manipulated to be deficient in both IL-12 and IL-23 or IL-12 alone *[see Nonclinical Toxicology (13)]*.

6 ADVERSE REACTIONS

The following serious adverse reactions are discussed elsewhere in the label:

- Infections *[see Warnings and Precautions (5.1)]*
- Malignancies *[see Warnings and Precautions (5.4)]*
- Reversible Posterior Leukoencephalopathy Syndrome *[see Warnings and Precautions (5.5)]*

6.1 Clinical Studies Experience

The safety data reflect exposure to STELARA™ in 2266 psoriasis subjects, including 1970 exposed for at least 6 months, 1285 exposed for at least one year, and 373 exposed for at least 18 months.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

Table 1 summarizes the adverse reactions that occurred at a rate of at least 1% and at a higher rate in the STELARA™ groups than the placebo group during the placebo-controlled period of STUDY 1 and STUDY 2.

Table 1. Adverse reactions reported by ≥1% of subjects through Week 12 in STUDY 1 and STUDY 2

	Placebo	STELARA™ 45 mg	STELARA™ 90 mg
Subjects treated	665	664	666
Nasopharyngitis	51 (8%)	56 (8%)	49 (7%)
Upper respiratory tract infection	30 (5%)	36 (5%)	28 (4%)
Headache	23 (3%)	33 (5%)	32 (5%)
Fatigue	14 (2%)	18 (3%)	17 (3%)
Diarrhea	12 (2%)	13 (2%)	13 (2%)
Back pain	8 (1%)	9 (1%)	14 (2%)
Dizziness	8 (1%)	8 (1%)	14 (2%)
Pharyngolaryngeal pain	7 (1%)	9 (1%)	12 (2%)
Pruritus	9 (1%)	10 (2%)	9 (1%)
Injection site erythema	3 (<1%)	6 (1%)	13 (2%)
Myalgia	4 (1%)	7 (1%)	8 (1%)
Depression	3 (<1%)	8 (1%)	4 (1%)

Adverse drug reactions that occurred at rates less than 1% included: cellulitis and certain injection site reactions (pain, swelling, pruritus, induration, hemorrhage, bruising, and irritation). One case of RPLS occurred during clinical trials *[see Warnings and Precautions (5.5)]*.

Infections

In the placebo-controlled period of clinical studies of psoriasis subjects (average follow-up of 12.6 weeks for placebo-treated subjects and 13.4 weeks for STELARA™-treated subjects), 27% of STELARA™-treated subjects reported infections (1.39 per subject-year of follow-up) compared with 24% of placebo-treated subjects (1.21 per subject-year of follow-up). Serious infections occurred in 0.3% of STELARA™-treated subjects (0.01 per subject-year of follow-up) and in 0.4% of placebo-treated subjects (0.02 per subject-year of follow-up) *[see Warnings and Precautions (5.1)]*.

In the controlled and non-controlled portions of psoriasis clinical trials, 61% of STELARA™-treated subjects reported infections (1.24 per subject-year of follow-up). Serious infections were reported in 0.9% of subjects (0.01 per subject-year of follow-up).

Malignancies

In the controlled and non-controlled portions of psoriasis clinical trials, 0.4% of STELARA™-treated subjects reported malignancies excluding non-melanoma skin cancers (0.36 per 100 subject-years of follow-up). Non-melanoma skin cancer was reported in 0.8% of STELARA™-treated subjects (0.80 per 100 subject-years of follow-up) *[see Warnings and Precautions (5.4)]*.

Serious malignancies included breast, colon, head and neck, kidney, prostate, and thyroid cancers.

Immunogenicity

The presence of ustekinumab in the serum can interfere with the detection of anti-ustekinumab antibodies resulting in inconclusive results due to assay interference. In STUDIES 1 and 2, antibody testing was done at time points when ustekinumab may have been present in the serum. Table 2 summarizes the antibody results from STUDIES 1 and 2. In STUDY 1 the last ustekinumab injection was between Weeks 28 and 48 and the last test for anti-ustekinumab antibodies was at Week 52. In STUDY 2 the last ustekinumab injection was at Week 16 and the last test for anti-ustekinumab antibodies was at Week 24.

Table 2

Antibody Results	**STUDY 1 (N=743)**	**STUDY 2 (N=1198)**
Positive	38 (5%)	33 (3%)
Negative	351 (47%)	90 (8%)
Inconclusive	354 (48%)	1075 (90%)

The data reflect the percentage of subjects whose test results were positive for antibodies to ustekinumab in a bridging immunoassay, and are highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody positivity in an assay may be influenced by several factors, including sample handling, timing of sample collection, concomitant medications and underlying disease. For these reasons, comparison of the incidence of antibodies to ustekinumab with the incidence of antibodies to other products may be misleading.

7 DRUG INTERACTIONS

Drug interaction studies have not been conducted with STELARA™.

7.1 Live Vaccines

Live vaccines should not be given concurrently with STELARA™ *[see Warnings and Precautions (5.5)]*.

7.2 Concomitant Therapies

The safety of STELARA™ in combination with immunosuppressive agents or phototherapy has not been evaluated *[see Warnings and Precautions (5.6)]*.

7.3 CYP450 Substrates

The formation of CYP450 enzymes can be altered by increased levels of certain cytokines (e.g., IL-1, IL-6, IL-10, TNFα, IFN) during chronic inflammation. Thus, ustekinumab could normalize the formation of CYP450 enzymes. A role for IL-12 or IL-23 in the regulation of CYP450 enzymes has not been reported. However, upon initiation of ustekinumab in patients who are receiving concomitant CYP450 substrates, particularly those with a narrow therapeutic index, monitoring for therapeutic effect (e.g., for warfarin) or drug concentration (e.g., for cyclosporine) should be considered and the individual dose of the drug adjusted as needed.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category B

There are no studies of STELARA™ in pregnant women. STELARA™ should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. No teratogenic effects were observed in the developmental and reproductive toxicology studies performed in cynomolgus monkeys at doses up to 45 mg/kg ustekinumab, which is 45 times (based on mg/kg) the highest intended clinical dose in psoriasis patients (approximately 1 mg/kg based on administration of a 90 mg dose to a 90 kg psoriasis patient).

Ustekinumab was tested in two embryo-fetal development toxicity studies. Pregnant cynomolgus monkeys were administered ustekinumab at doses up to 45 mg/kg during the period of organogenesis either twice weekly via subcutaneous injections or weekly by intravenous injections. No significant adverse developmental effects were noted in either study.

In an embryo-fetal development and pre- and post-natal development toxicity study, three groups of 20 pregnant cynomolgus monkeys were administered subcutaneous doses of 0, 22.5, or 45 mg/kg ustekinumab twice weekly from the beginning of organogenesis in cynomolgus monkeys to Day 33 after delivery. There were no treatment-related effects on mortality, clinical signs, body weight, food consumption, hematology, or serum biochemistry in dams. Fetal losses occurred in six control monkeys, six 22.5 mg/kg-treated monkeys, and five 45 mg/kg-treated monkeys. Neonatal deaths occurred in one 22.5 mg/kg-treated monkey and in one 45 mg/kg-treated monkey. No ustekinumab-related abnormalities were observed in the neonates from birth through six months of age in clinical signs, body weight, hematology, or serum biochemistry. There were no treatment-related effects on functional development until weaning, functional development after weaning, morphological development, immunological development, and gross and histopathological examinations of offsprings by the age of 6 months.

8.3 Nursing Mothers

Caution should be exercised when STELARA™ is administered to a nursing woman. The unknown risks to the infant from gastrointestinal or systemic exposure to ustekinumab should be weighed against the known benefits of breast-feeding. Ustekinumab is excreted in the milk of lactating monkeys administered ustekinumab. IgG is excreted in human milk, so it is expected that STELARA™ will be present in human milk. It is not known if ustekinumab is absorbed systemically after ingestion; however, published data suggest that antibodies in breast milk do not enter the neonatal and infant circulation in substantial amounts.

8.4 Pediatric Use

Safety and effectiveness of STELARA™ in pediatric patients have not been evaluated.

8.5 Geriatric Use

Of the 2266 psoriasis subjects exposed to STELARA™, a total of 131 were 65 years or older, and 14 subjects were 75 years or older. Although no differences in safety or efficacy were observed between older and younger subjects, the number of subjects aged 65 and over is not sufficient to determine whether they respond differently from younger subjects.

10 OVERDOSAGE

Single doses up to 4.5 mg/kg intravenously have been administered in clinical studies without dose-limiting toxicity. In case of overdosage, it is recommended that the patient be monitored for any signs

or symptoms of adverse reactions or effects and appropriate symptomatic treatment be instituted immediately.

11 DESCRIPTION

STELARA™ is a human IgG1κ monoclonal antibody against the p40 subunit of the IL-12 and IL-23 cytokines. Using DNA recombinant technology, STELARA™ is produced in a well characterized recombinant cell line and is purified using standard bio-processing technology. The manufacturing process contains steps for the clearance of viruses. STELARA™ is comprised of 1326 amino acids and has an estimated molecular mass that ranges from 148,079 to 149,690 Daltons.

STELARA™ is available as: 45 mg of ustekinumab in 0.5 mL and 90 mg of ustekinumab in 1 mL. STELARA™ is supplied as a sterile solution in a single-use 2 mL Type I stoppered glass vial with a coated stopper. The STELARA™ solution is colorless to slightly yellow in appearance and has a pH of 5.7-6.3.

The 45 mg vial contains: 45 mg ustekinumab, 38 mg sucrose, 0.5 mg L-histidine, and 0.02 mg Polysorbate 80 to fill to a final volume of 0.5 mL.

The 90 mg vial contains: 90 mg ustekinumab, 76 mg sucrose, 1 mg L-histidine, and 0.04 mg Polysorbate 80 to fill to a final volume of 1 mL.

STELARA™ does not contain preservatives.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Ustekinumab is a human IgG1κ monoclonal antibody that binds with high affinity and specificity to the p40 protein subunit used by both the interleukin (IL)-12 and IL-23 cytokines. IL-12 and IL-23 are naturally occurring cytokines that are involved in inflammatory and immune responses, such as natural killer cell activation and CD4+ T-cell differentiation and activation. In *in vitro* models, ustekinumab was shown to disrupt IL-12 and IL-23 mediated signaling and cytokine cascades by disrupting the interaction of these cytokines with a shared cell-surface receptor chain, IL-12 β1.

12.2 Pharmacodynamics

In a small exploratory study, a decrease was observed in the expression of mRNA of its molecular targets IL-12 and IL-23 in lesional skin biopsies measured at baseline and up to two weeks post-treatment in psoriatic subjects.

12.3 Pharmacokinetics

Absorption

In psoriasis subjects, the median time to reach the maximum serum concentration (T_{max}) was 13.5 days and 7 days, respectively, after a single subcutaneous administration of 45 mg (N=22) and 90 mg (N=24) of ustekinumab. In healthy subjects (N=30), the median T_{max} value (8.5 days) following a single subcutaneous administration of 90 mg of ustekinumab was comparable to that observed in psoriasis subjects. Following multiple subcutaneous doses of STELARA™, the steady-state serum concentrations of ustekinumab were achieved by Week 28. The mean (±SD) steady-state trough serum concentration ranged from 0.31 ± 0.33 mcg/mL (45 mg) to 0.64 ± 0.64 mcg/mL (90 mg). There was

no apparent accumulation in serum ustekinumab concentration over time when given subcutaneously every 12 weeks.

Distribution
Following subcutaneous administration of 45 mg (N=18) and 90 mg (N=21) of ustekinumab to psoriasis subjects, the mean (±SD) apparent volume of distribution during the terminal phase (Vz/F) was 161 ± 65 mL/kg and 179 ± 85 mL/kg, respectively. The mean (± SD) volume of distribution during the terminal phase (Vz) following a single intravenous administration to subjects with psoriasis ranged from 56.1 ± 6.5 to 82.1 ± 23.6 mL/kg.

Metabolism
The metabolic pathway of ustekinumab has not been characterized. As a human IgG1κ monoclonal antibody ustekinumab is expected to be degraded into small peptides and amino acids via catabolic pathways in the same manner as endogenous IgG.

Elimination
The mean (± SD) systemic clearance (CL) following a single intravenous administration of ustekinumab to psoriasis subjects ranged from 1.90 ± 0.28 to 2.22 ± 0.63 mL/day/kg. The mean (±SD) half-life ranged from 14.9 ± 4.6 to 45.6 ± 80.2 days across all psoriasis studies following intravenous and subcutaneous administration.

Weight
When given the same dose, subjects weighing >100 kg had lower median serum ustekinumab concentrations compared with those subjects weighing ≤100 kg.

Hepatic and Renal Impairment
No pharmacokinetic data are available in patients with hepatic or renal impairment.

Elderly
A population pharmacokinetic analysis (N=106/1937 subjects greater than or equal to 65 years old) was performed to evaluate the effect of age on the pharmacokinetics of ustekinumab. There were no apparent changes in pharmacokinetic parameters (clearance and volume of distribution) in subjects older than 65 years old.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Animal studies have not been conducted to evaluate the carcinogenic or mutagenic potential of STELARA™. Published literature showed that administration of murine IL-12 caused an anti-tumor effect in mice that contained transplanted tumors and IL-12/IL-23p40 knockout mice or mice treated with anti-IL-12/IL-23p40 antibody had decreased host defense to tumors. Mice genetically manipulated to be deficient in both IL-12 and IL-23 or IL-12 alone developed UV-induced skin cancers earlier and more frequently compared to wild-type mice. The relevance of these experimental findings in mouse models for malignancy risk in humans is unknown.

A male fertility study was conducted with only 6 male monkeys per group administered subcutaneous doses of 0, 22.5, or 45 mg/kg ustekinumab twice weekly prior to mating and during the mating period for 13 weeks, followed by a 13-week treatment-free period. Although fertility and pregnancy

outcomes were not evaluated in mated females, there were no treatment-related effects on parental toxicity or male fertility parameters.

A female fertility study was conducted in mice using an analogous IL-12/IL-23p40 antibody by subcutaneous administration at doses up to 50 mg/kg, twice weekly, beginning 15 days before cohabitation and continuing through GD 7. There were no treatment-related effects on maternal toxicity or female fertility parameters.

13.2 Animal Toxicology and/or Pharmacology
In a 26-week toxicology study, one out of 10 monkeys subcutaneously administered 45 mg/kg ustekinumab twice weekly for 26 weeks had a bacterial infection.

14 CLINICAL STUDIES

Two multicenter, randomized, double-blind, placebo-controlled studies (STUDY 1 and STUDY 2) enrolled a total of 1996 subjects 18 years of age and older with plaque psoriasis who had a minimum body surface area involvement of 10%, and Psoriasis Area and Severity Index (PASI) score $\geq$12, and who were candidates for phototherapy or systemic therapy. Subjects with guttate, erythrodermic, or pustular psoriasis were excluded from the studies.

STUDY 1 enrolled 766 subjects and STUDY 2 enrolled 1230 subjects. The studies had the same design through Week 28. In both studies, subjects were randomized in equal proportion to placebo, 45 mg or 90 mg of STELARA™. Subjects randomized to STELARA™ received 45 mg or 90 mg doses, regardless of weight, at Weeks 0, 4, and 16. Subjects randomized to receive placebo at Weeks 0 and 4 crossed over to receive STELARA™ (either 45 mg or 90 mg) at Weeks 12 and 16.

In both studies, the endpoints were the proportion of subjects who achieved at least a 75% reduction in PASI score (PASI 75) from baseline to Week 12 and treatment success (cleared or minimal) on the Physician's Global Assessment (PGA). The PGA is a 6-category scale ranging from 0 (cleared) to 5 (severe) that indicates the physician's overall assessment of psoriasis focusing on plaque thickness/induration, erythema, and scaling.

In both studies, subjects in all treatment groups had a median baseline PASI score ranging from approximately 17 to 18. Baseline PGA score was marked or severe in 44% of subjects in STUDY 1 and 40% of subjects in STUDY 2. Approximately two-thirds of all subjects had received prior phototherapy, 69% had received either prior conventional systemic or biologic therapy for the treatment of psoriasis, with 56% receiving prior conventional systemic therapy and 43% receiving prior biologic therapy. A total of 28% of study subjects had a history of psoriatic arthritis.

Clinical Response
The results of STUDY 1 and STUDY 2 are presented in Table 3 below.

Table 3. Clinical Outcomes STUDY 1 and STUDY 2

Week 12	STUDY 1			STUDY 2		
		STELARA™			STELARA™	
	Placebo	45 mg	90 mg	Placebo	45 mg	90 mg
Subjects randomized	255	255	256	410	409	411
PASI 75 response	8 (3%)	171 (67%)	170 (66%)	15 (4%)	273 (67%)	311 (76%)
PGA of Cleared or Minimal	10 (4%)	151 (59%)	156 (61%)	18 (4%)	277 (68%)	300 (73%)

Examination of age, gender, and race subgroups did not identify differences in response to STELARA™ among these subgroups.

In subjects who weighed <100 kg, response rates were similar with both the 45 mg and 90 mg doses; however, in subjects who weighed >100 kg, higher response rates were seen with 90 mg dosing compared with 45 mg dosing (Table 4 below).

Table 4. Clinical Outcomes by Weight STUDY 1 and STUDY 2

	STUDY 1			STUDY 2		
		STELARA™			STELARA™	
	Placebo	45 mg	90 mg	Placebo	45 mg	90 mg
Subjects randomized	255	255	256	410	409	411
Week 12						
PASI 75 response						
≤100 kg	4%	74%	65%	4%	73%	78%
	6/166	124/168	107/164	12/290	218/297	225/289
>100 kg	2%	54%	68%	3%	49%	71%
	2/89	47/87	63/92	3/120	55/112	86/121
PGA of Cleared or Minimal						
≤100 kg	4%	64%	63%	5%	74%	75%
	7/166	108/168	103/164	14/290	220/297	216/289
>100 kg	3%	49%	58%	3%	51%	69%
	3/89	43/87	53/92	4/120	57/112	84/121

Subjects in STUDY 1 were evaluated through Week 52. At Week 40, those who were PASI 75 responders at both Weeks 28 and 40 were re-randomized to either continued dosing of STELARA™ (STELARA™ at Week 40) or to withdrawal of therapy (placebo at Week 40). At Week 52, 89% (144/162) of subjects re-randomized to STELARA™ treatment were PASI 75 responders compared with 63% (100/159) of subjects re-randomized to placebo (treatment withdrawal after Week 28 dose).

16 HOW SUPPLIED/STORAGE AND HANDLING

STELARA™ does not contain preservatives. STELARA™ is available in single-use vials containing 45 mg or 90 mg of ustekinumab.

The NDC number for the 45 mg vial is 57894-060-02.

The NDC number for the 90 mg vial is 57894-061-02.

Storage and Stability
Store STELARA™ upright and refrigerated at 2°C to 8°C (36°F to 46°F). Keep the product in the original carton to protect from light until the time of use. Do not freeze. Do not shake. STELARA™ does not contain a preservative; discard any unused portion.

17 PATIENT COUNSELING INFORMATION
Instruct patients to read the Medication Guide before starting STELARA™ therapy and to reread the Medication Guide each time the prescription is renewed.

Infections
Inform patients that STELARA™ may lower the ability of their immune system to fight infections. Instruct patients of the importance of communicating any history of infections to the doctor, and contacting their doctor if they develop any symptoms of infection.

Malignancies
Patients should be counseled about the risk of malignancies while receiving STELARA™.

Manufactured by Cilag AG,
Schaffhausen, Switzerland
for
Centocor Ortho Biotech Inc.
Horsham, PA 19044
U.S. License No. 1821

MEDICATION GUIDE
STELARA™ (stel ar' a)
(ustekinumab)
Injection

Read this Medication Guide before you start taking STELARA™ and each time before you get an injection. There may be new information. This Medication Guide does not take the place of talking with your doctor about your medical condition or treatment with STELARA™.

What is the most important information I should know about STELARA™?

STELARA™ is a medicine that affects your immune system. STELARA™ can increase your chances of having serious side effects, including:

Serious Infections: STELARA™ may lower the ability of your immune system to fight infections and may increase your risk of infections. Some people have serious infections while taking STELARA™, including tuberculosis (TB), and infections caused by bacteria, fungi, or viruses. Some people have to be hospitalized for treatment of their infection.

- Your doctor should check you for TB before starting STELARA™.
- If your doctor feels that you are at risk for TB, you may be treated with medicine for TB before you begin treatment with STELARA™ and during treatment with STELARA™.
- Your doctor should watch you closely for signs and symptoms of TB during treatment with STELARA™.

You should not start taking STELARA™ if you have any kind of infection unless your doctor says it is okay.

Before starting STELARA™, tell your doctor if you think you have an infection or have symptoms of an infection such as:

- fever, sweats, or chills
- muscle aches
- cough
- shortness of breath
- blood in your phlegm
- weight loss
- warm, red, or painful skin or sores on your body
- diarrhea or stomach pain

- burning when you urinate or urinate more often than normal
- feel very tired
- are being treated for an infection
- get a lot of infections or have infections that keep coming back
- have TB, or have been in close contact with someone who has TB.

After starting STELARA™, call your doctor right away if you have any symptoms of an infection (see above).

STELARA™ can make you more likely to get infections or make an infection that you have worse.

People who have a genetic problem where the body does not make any of the proteins interleukin 12 (IL-12) and interleukin 23 (IL-23) are at a higher risk for certain serious infections. These infections can spread throughout the body and cause death. It is not known if people who take STELARA™ will get any of these infections, because of the effects of STELARA™ on these proteins in your body.

Cancers:

STELARA™ may decrease the activity of your immune system and increase your risk for certain types of cancers. Tell your doctor if you have ever had any type of cancer.

Reversible Posterior Leukoencephalopathy Syndrome (RPLS):

RPLS is a rare condition that affects the brain and can cause death. The cause of RPLS is not known. If RPLS is found early and treated, most people recover. Tell your doctor right away if you have any new or worsening medical problems including:

- headache
- seizures
- confusion
- vision problems

What is STELARA™?

STELARA™ is a prescription medicine used to treat adults 18 years and older with moderate or severe psoriasis that involves large areas or many areas of their body, who may benefit from taking injections or pills (systemic therapy) or phototherapy (treatment using ultraviolet light alone or with pills).

STELARA™ may improve your psoriasis but may also lower the ability of your immune system to fight infections. This may also increase your risk for certain types of cancer.

It is not known if STELARA™ is safe and effective in children.

It is not known if taking STELARA™ for more than 2 years is safe and effective.

What should I tell my doctor before receiving STELARA™?

Before receiving STELARA™, tell your doctor if you:

- have any of the conditions or symptoms listed in the section "What is the most important information I should know about STELARA™?"
- have recently received or are scheduled to receive an immunization (vaccine). People who take STELARA™ should not receive live vaccines. Tell your doctor if anyone in your house needs a vaccine. The viruses used in some types of vaccines can spread to people with a weakened immune system, and can cause serious problems. **You should not receive the BCG vaccine during the one year before taking STELARA™ or one year after you stop taking STELARA™.**
- receive phototherapy for your psoriasis.
- have any other medical conditions.
- are pregnant or planning to become pregnant. It is not known if STELARA™ will harm your unborn baby. You and your doctor should decide if you will take STELARA™.
- are breast-feeding or plan to breast-feed. It is thought that STELARA™ passes into your breast milk. You should not breast-feed while taking STELARA™ without first talking with your doctor.

Tell your doctor about all the medicines you take, including prescription and non-prescription medicines, vitamins, and herbal supplements. Especially tell your doctor if you take:

- other medicines that affect your immune system.
- certain medicines that can affect how your liver breaks down other medicines.

Ask your doctor or pharmacist if you are not sure if your medicine is one that is listed above.

Know the medicines you take. Keep a list of them to show your doctor and pharmacist when you get a new medicine.

How will I receive STELARA™?

- STELARA™ is given by injection under the skin (subcutaneous injection).
- STELARA™ should only be given by a healthcare provider as directed by your doctor.
- Your doctor will decide the right dose of STELARA™ for you and how often you should receive it.
- Be sure to keep all of your scheduled follow-up appointments.

What should I avoid while receiving STELARA™?

You should not receive a live vaccine while taking STELARA™. See "What should I tell my doctor before taking STELARA™?"

What are the possible side effects of STELARA™?

STELARA™ can increase your chances of having serious side effects. See "What is the most important information I should know about STELARA™?"

Common side effects of STELARA™ include:

- upper respiratory infections
- headache
- tiredness

These are not all of the possible side effects of STELARA™. Tell your doctor about any side effect that bothers you or that does not go away. For more information, ask your doctor or pharmacist.

Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

General information about STELARA™

Medicines are sometimes prescribed for purposes other than those listed in a Medication Guide.

This Medication Guide summarizes the most important information about STELARA™. If you would like more information, talk with your doctor. You can ask your doctor or pharmacist for information about STELARA™ that was written for healthcare professionals. For more information, go to www.STELARAinfo.com or call 1-800-457-6399.

What are the ingredients in STELARA™?

Active ingredient: ustekinumab

Inactive ingredients: sucrose, L-histidine, and polysorbate 80.

Manufactured by Cilag AG,
Schaffhausen, Switzerland
for
Centocor Ortho Biotech Inc.
Horsham, PA 19044

Revised September 2009

This Medication Guide has been approved by the U.S. Food and Drug Administration.

U.S. License No. 1821

45 mg version - Enlarged 300%
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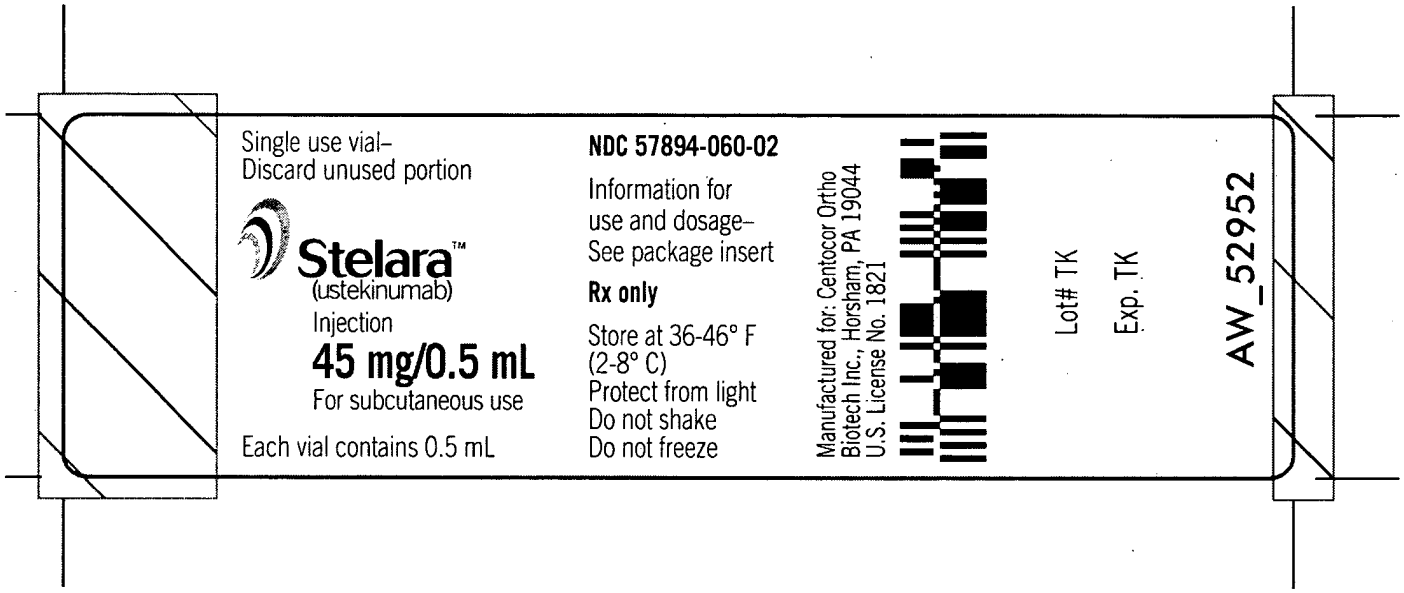

45 mg version - 100%
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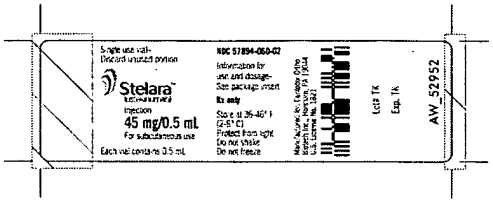

GPSG Global Pharmaceutical Supply Group

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MODIFIED DATE 06/18/09	DESIGNER Kim DePaola

Date/Comment: TITLE BLOCK PROOFED ☐ NO ■ YES

04/14/09: V1: CR created from 10139900 with revs per PDF.
06/18/09: V2: KD made revs per marked up PDF.

COLORS USED IN FILE

BLACK | PMS 2607 | PMS 3242 | PMS 605 | Security LPI-1013 | Non Varnish Indication

DIMENSIONS 2-13/64" x 21/32"

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SPEC # 81877 ePM Order # 52952

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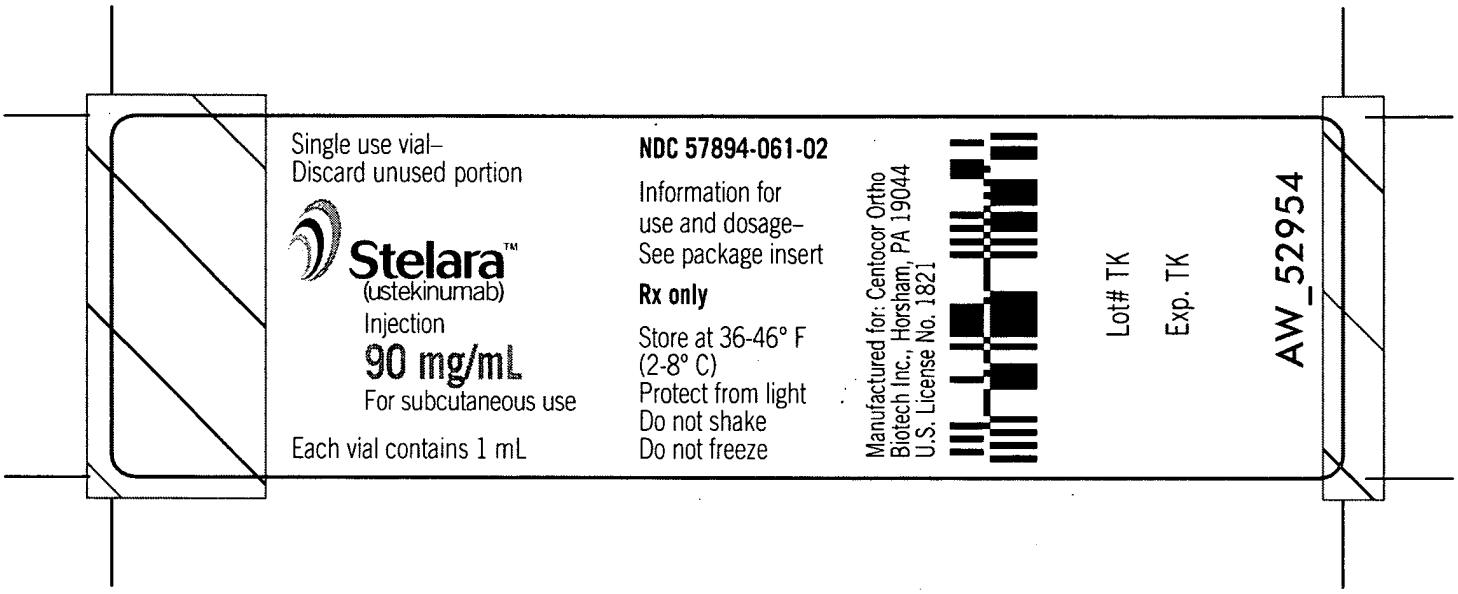

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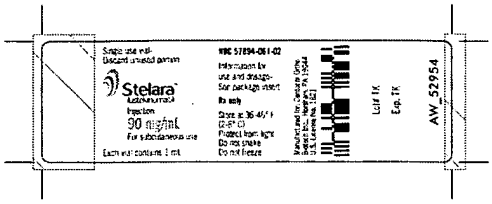

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GPSG Global Pharmaceutical Supply Group

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SOFTWARE USED Illustrator	**VERS.** CS2 **UPGRADE** ■
MODIFIED DATE 06/18/09	**DESIGNER** Kim DePaola
Date/Comment:	**TITLE BLOCK PROOFED** ☐ NO ■ YES

04/14/09: V1: CR created from 10139900 with revs per PDF.
06/18/09: V2: KD made revs per marked up PDF

COLORS USED IN FILE

BLACK PMS 2607 PMS 3242 PMS 605 Security LPI-1013 Non Varnish Indication

DIMENSIONS 2-13/64" x 21/32"

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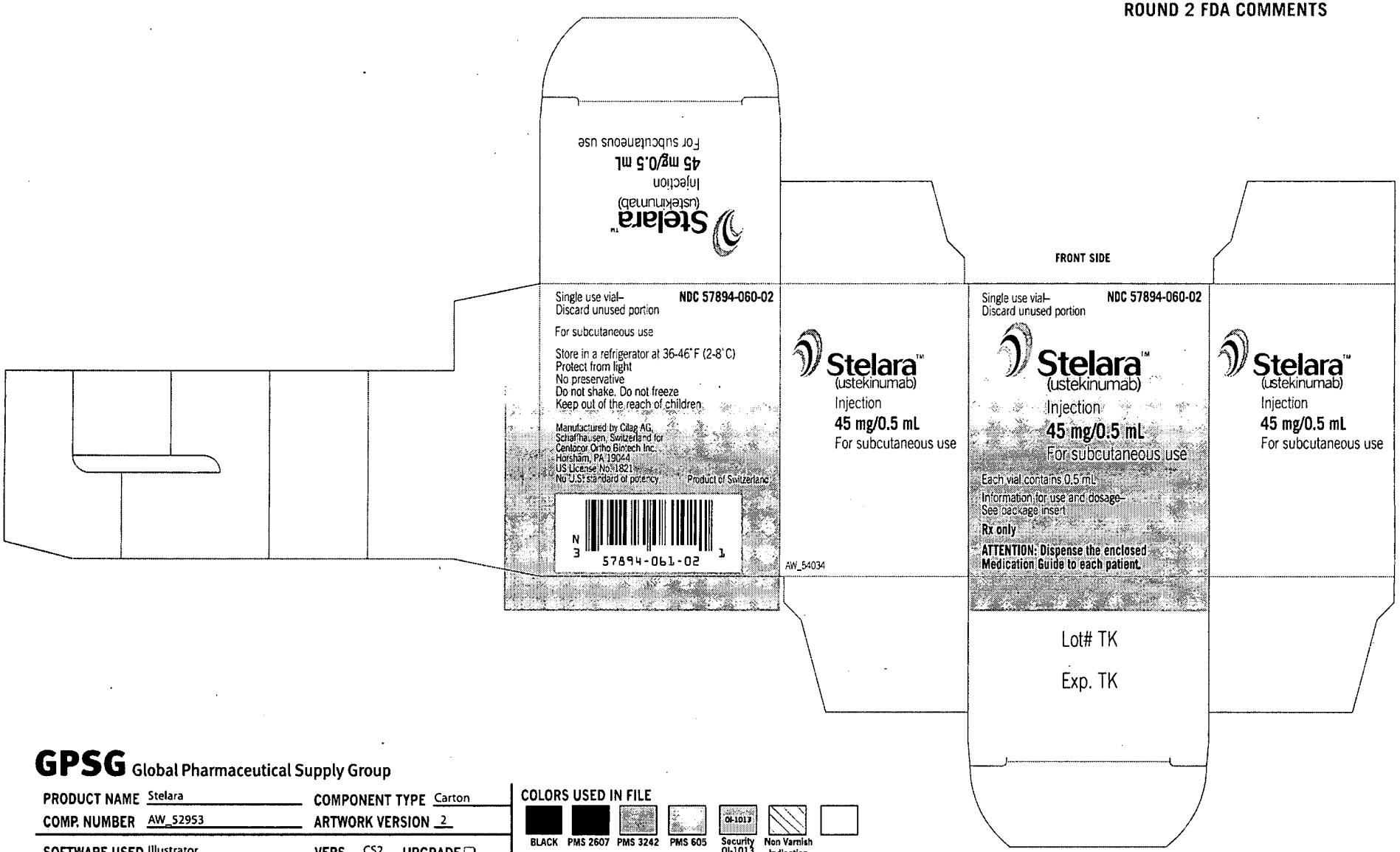

GPSG Global Pharmaceutical Supply Group

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SOFTWARE USED Illustrator	VERS. CS2 UPGRADE ☐
MODIFIED DATE 06/18/09	DESIGNER Kim DePaola
Date/Comment:	TITLE BLOCK PROOFED ☐ NO ■ YES

COLORS USED IN FILE

BLACK | PMS 2607 | PMS 3242 | PMS 605 | Security OI-1013 | Non Varnish Indication

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DWG # NA OUTPUT % 100%

SPEC # NA ePM Order # 52953

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06/18/09: V2: KD used die and layout from AW_54034 per LS and made changes per marked up PDF

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FDA COMMENTS- RND 2

PMS Orange 021

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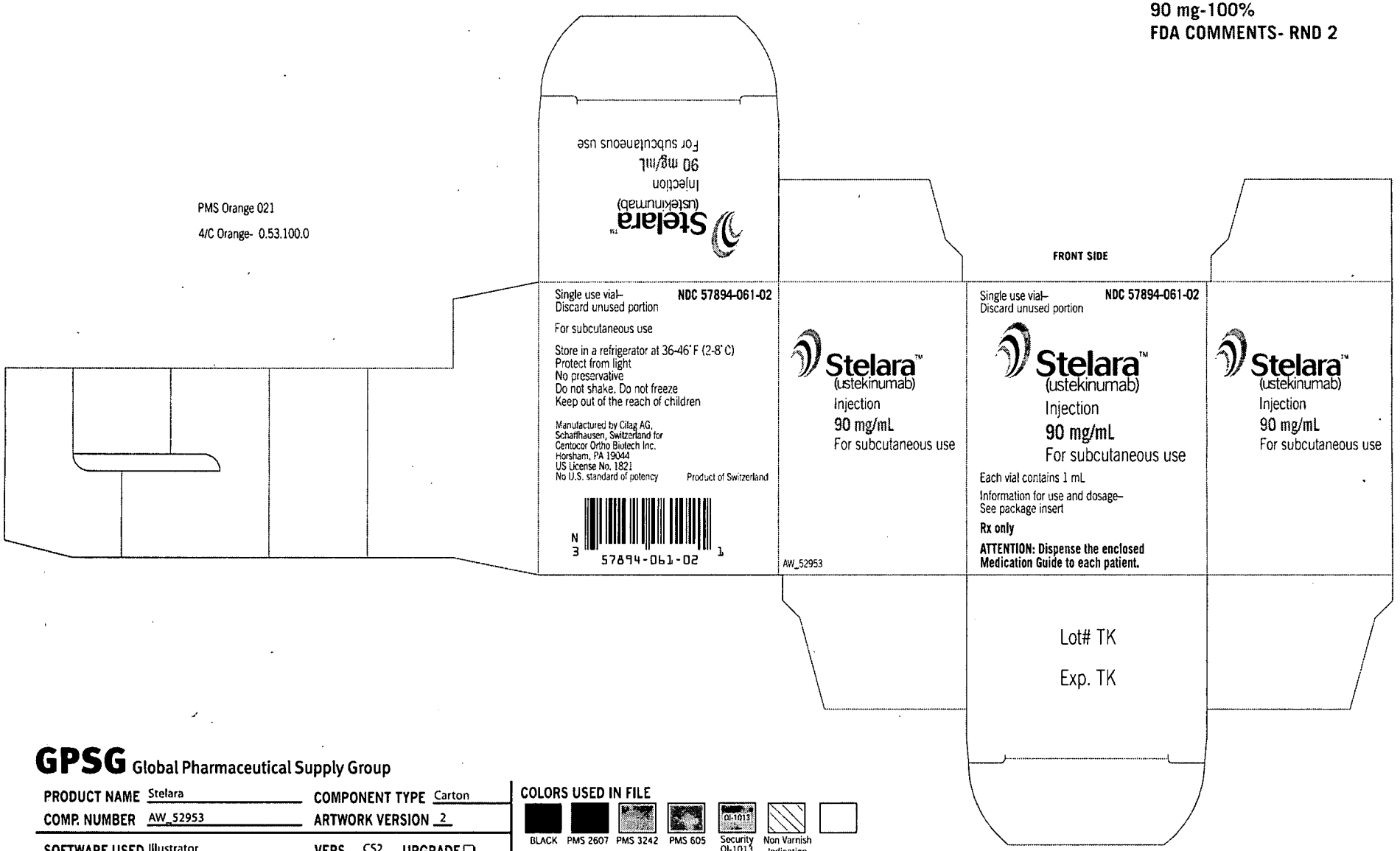

GPSG Global Pharmaceutical Supply Group

PRODUCT NAME Stelara	COMPONENT TYPE Carton
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MODIFIED DATE 06/18/09	DESIGNER Kim DePaola
Date/Comment:	TITLE BLOCK PROOFED ☐ NO ■ YES

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SPEC # NA ePM Order # 52953

04/14/09: V1: CR created document from 10139701 with changes per PDF
06/18/09: V2: KD used die and layout from AW_54034 per LS and made changes per marked up PDF

Appendix A. REMS Document

BLA 125261 STELARA™ (ustekinumab)

Human interleukin 12 and 23 antagonist

RISK EVALUATION AND MITIGATION STRATEGY (REMS)

I. GOALS

To evaluate and mitigate the potential risks of serious infections and malignancy, and reversible posterior leukoencephalopathy syndrome (RPLS) associated with STELARA™ by:

- alerting and warning healthcare providers and patients about the risks
- informing and educating healthcare providers about the Psoriasis Longitudinal Assessment and Registry (PSOLAR) voluntary disease-specific patient registry

II. REMS ELEMENTS

A. MEDICATION GUIDE

In accordance with 21 CFR 208.24, a Medication Guide will be appended to the package insert and will be provided by Centocor Ortho Biotech Inc. and/or its affiliates by either:

1. Providing Medication Guides in sufficient numbers to distributors, packers, or authorized dispensers to permit the authorized dispenser to provide a Medication Guide to each patient receiving a prescription for the drug product; or
2. Providing the means to produce Medication Guides in sufficient numbers to distributors, packers, or authorized dispensers to permit the authorized dispenser to provide a Medication Guide to each patient receiving a prescription for the drug product.

Medication Guides will be included in each single unit carton containing one vial and dispensed in accordance with 21 CFR 208.24.

The Medication Guide will also be available on the STELARA™ patient and professional websites.

Please see appended Medication Guide (Attachment A).

B. COMMUNICATION PLAN

Centocor Ortho Biotech Inc. will implement a communication plan to the following healthcare providers:

- Dermatologists and dermatology healthcare providers (HCPs) who are likely to prescribe and/or inject STELARA™

- Oncologists who may treat malignancies potentially associated with the use of immunosuppressants for chronic inflammatory and autoimmune disease and will need to ascertain cases of malignancy after a long latency period

- Rheumatologists who may co-manage with dermatologists moderate to severe psoriasis patients with psoriatic arthritis

- Infectious disease specialists and gastroenterologists who may be consulted about infections and will need to understand the potential for infectious complications of IL-12/IL-23 blockade

- Neurologists who may treat RPLS

The communication plan will provide for the dissemination of risk information about serious infection, malignancy, and RPLS, and encourage patient participation and physician referral to PSOLAR investigator sites (a voluntary psoriasis registry).

Elements of the communication plan:

1. A Dear Healthcare Professional letter (see Attachment B) will be distributed to dermatologists, oncologists, rheumatologists, infectious disease specialists, gastroenterologists, and neurologists. This will be distributed within 60 days of STELARA™ approval.

   A Professional Label and a copy of the Medication Guide will also be distributed in this communication.

2. A Dear Pharmacist letter (see Attachment C) will be distributed to pharmacists. This will be distributed within 60 days of STELARA™ approval.

   A Professional Label and a copy of the Medication Guide will also be distributed in this communication.

3. Dissemination of information about serious infection, malignancy, and RPLS to health care providers through certain dermatology, oncology, rheumatology, infectious diseases, and gastroenterology professional societies' journals:

   1) For display as a panel/poster and distribution as printed material at all dermatology and oncology scientific meetings where the company has a sponsored booth

   2) For quarterly presentation as a printed information piece in the *Journal of the American Academy of Dermatology* and the *Archives of Dermatology* for 3 years

3) For quarterly presentation as a printed information piece in the *Journal of Clinical Oncology* and *Blood* for 5 years

4) For twice yearly presentation as a printed information piece in *Arthritis and Rheumatism,* the *Journal of Infectious Disease,* the *American Journal of Gastroenterology,* and *Gastroenterology* for 3 years

The REMS Journal Information Piece is appended to this document (see Attachments D, E, F, G, and H).

4. Centocor Ortho Biotech Inc. will enhance participation in PSOLAR, a voluntary, disease-specific registry (which is comprised of patients receiving systemic therapies including other biologic agents), by outreach to dermatologic societies, inclusion of PSOLAR contact information in communication materials, and proactively educating dermatologists about PSOLAR.

C. TIMETABLE FOR SUBMISSION OF ASSESSMENTS

Centocor Ortho Biotech Inc. will submit REMS Assessments to the FDA according to the table below. To facilitate inclusion of as much information as possible while allowing reasonable time to prepare the submission, the reporting interval covered by each assessment should conclude no earlier than 60 days before the submission date for that assessment. Centocor Ortho Biotech Inc. will submit each assessment so that it will be received by the FDA on or before the due date.

Assessment Submission	**Timing Interval Relative to Approval of the REMS**	**Date of Submission to the FDA (month/year)**
1st Assessment	18 months after approval	March, 2011
2nd Assessment	3 years after approval	September, 2012
3rd Assessment	7 years after approval	September, 2016

Attachment A Medication Guide

MEDICATION GUIDE

STELARA™ (stel ar' a)

(ustekinumab)

Injection

Read this Medication Guide before you start taking STELARA™ and each time before you get an injection. There may be new information. This Medication Guide does not take the place of talking with your doctor about your medical condition or treatment with STELARA™.

What is the most important information I should know about STELARA™?

STELARA™ is a medicine that affects your immune system. STELARA™ can increase your chances of having serious side effects, including:

Serious Infections: STELARA™ may lower the ability of your immune system to fight infections and may increase your risk of infections. Some people have serious infections while taking STELARA™, including tuberculosis (TB), and infections caused by bacteria, fungi, or viruses. Some people have to be hospitalized for treatment of their infection.

- Your doctor should check you for TB before starting STELARA™.
- If your doctor feels that you are at risk for TB, you may be treated with medicine for TB before you begin treatment with STELARA™ and during treatment with STELARA™.
- Your doctor should watch you closely for signs and symptoms of TB during treatment with STELARA™.

You should not start taking STELARA™ if you have any kind of infection unless your doctor says it is okay.

Before starting STELARA™, tell your doctor if you think you have an infection or have symptoms of an infection such as:

- fever, sweats, or chills
- muscle aches
- cough
- shortness of breath
- blood in your phlegm
- weight loss

- warm, red, or painful skin or sores on your body
- diarrhea or stomach pain
- burning when you urinate or urinate more often than normal
- feel very tired
- are being treated for an infection
- get a lot of infections or have infections that keep coming back
- have TB, or have been in close contact with someone who has TB.

After starting STELARA™, call your doctor right away if you have any symptoms of an infection (see above).

STELARA™ can make you more likely to get infections or make an infection that you have worse.

People who have a genetic problem where the body does not make any of the proteins interleukin 12 (IL-12) and interleukin 23 (IL-23) are at a higher risk for certain serious infections. These infections can spread throughout the body and cause death. It is not known if people who take STELARA™ will get any of these infections, because of the effects of STELARA™ on these proteins in your body.

Cancers:

STELARA™ may decrease the activity of your immune system and increase your risk for certain types of cancers. Tell your doctor if you have ever had any type of cancer.

Reversible Posterior Leukoencephalopathy Syndrome (RPLS):

RPLS is a rare condition that affects the brain and can cause death. The cause of RPLS is not known. If RPLS is found early and treated, most people recover. Tell your doctor right away if you have any new or worsening medical problems including:

- headache
- seizures
- confusion
- vision problems

What is STELARA™?

STELARA™ is a prescription medicine used to treat adults 18 years and older with moderate or severe psoriasis that involves large areas or many areas of their body, who may benefit from taking injections or pills (systemic therapy) or phototherapy (treatment using ultraviolet light alone or with pills).

STELARA™ may improve your psoriasis but may also lower the ability of your immune system to fight infections. This may also increase your risk for certain types of cancer.

It is not known if STELARA™ is safe and effective in children.

It is not known if taking STELARA™ for more than 2 years is safe and effective.

What should I tell my doctor before receiving STELARA™?

Before receiving STELARA™, tell your doctor if you:

- have any of the conditions or symptoms listed in the section "What is the most important information I should know about STELARA™?"
- have recently received or are scheduled to receive an immunization (vaccine). People who take STELARA™ should not receive live vaccines. Tell your doctor if anyone in your house needs a vaccine. The viruses used in some types of vaccines can spread to people with a weakened immune system, and can cause serious problems. **You should not receive the BCG vaccine during the one year before taking STELARA™ or one year after you stop taking STELARA™.**
- receive phototherapy for your psoriasis.
- have any other medical conditions.
- are pregnant or planning to become pregnant. It is not known if STELARA™ will harm your unborn baby. You and your doctor should decide if you will take STELARA™.
- are breast-feeding or plan to breast-feed. It is thought that STELARA™ passes into your breast milk. You should not breast-feed while taking STELARA™ without first talking with your doctor.

Tell your doctor about all the medicines you take, including prescription and non-prescription medicines, vitamins, and herbal supplements. Especially tell your doctor if you take:

- other medicines that affect your immune system.
- certain medicines that can affect how your liver breaks down other medicines.

Ask your doctor or pharmacist if you are not sure if your medicine is one that is listed above.

Know the medicines you take. Keep a list of them to show your doctor and pharmacist when you get a new medicine.

How will I receive STELARA™?

- STELARA™ is given by injection under the skin (subcutaneous injection).
- STELARA™ should only be given by a healthcare provider as directed by your doctor.
- Your doctor will decide the right dose of STELARA™ for you and how often you should receive it.

- Be sure to keep all of your scheduled follow-up appointments.

What should I avoid while receiving STELARA™?

You should not receive a live vaccine while taking STELARA™. See "What should I tell my doctor before taking STELARA™?"

What are the possible side effects of STELARA™?

STELARA™ can increase your chances of having serious side effects. See "What is the most important information I should know about STELARA™?"

Common side effects of STELARA™ include:

- upper respiratory infections
- headache
- tiredness

These are not all of the possible side effects of STELARA™. Tell your doctor about any side effect that bothers you or that does not go away. For more information, ask your doctor or pharmacist.

Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

General information about STELARA™

Medicines are sometimes prescribed for purposes other than those listed in a Medication Guide.

This Medication Guide summarizes the most important information about STELARA™. If you would like more information, talk with your doctor. You can ask your doctor or pharmacist for information about STELARA™ that was written for healthcare professionals. For more information, go to www.STELARAinfo.com or call 1-800-457-6399.

What are the ingredients in STELARA™?

Active ingredient: ustekinumab

Inactive ingredients: sucrose, L-histidine, and polysorbate 80.

Manufactured by Cilag AG,
Schaffhausen, Switzerland
for

Centocor Ortho Biotech Inc.

Horsham, PA 19044

Revised September 2009

This Medication Guide has been approved by the U.S. Food and Drug Administration.

U.S. License No. 1821

Attachment B Dear Healthcare Professional Letter

Centocor Ortho Biotech Inc.

IMPORTANT DRUG WARNING

Dear Healthcare Professional:

The purpose of this letter is to inform you of important safety information for STELARA™ (ustekinumab), a new human monoclonal antibody which has been approved by the Food and Drug Administration (FDA) for the treatment of adult patients (18 years or older) with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy. STELARA™ targets interleukin-12 (IL-12) and interleukin-23 (IL-23). FDA has determined that a Risk Evaluation and Mitigation Strategy (REMS) is necessary for STELARA™ to ensure that the benefits of the drug outweigh the potential risks of serious infections and malignancy, and reversible posterior leukoencephalopathy syndrome (RPLS).

IMPORTANT SAFETY INFORMATION FOR POTENTIAL RISKS OF SERIOUS INFECTIONS AND MALIGNANCIES, AND RPLS

The following information is important for healthcare professionals and patients treated with STELARA™:

Infections

- STELARA™ may increase the risk of infections and reactivation of latent infections. Serious bacterial, fungal, and viral infections, some requiring hospitalization, were observed in patients receiving STELARA™. STELARA™ should not be given to patients with a clinically important active infection and should not be administered until the infection resolves or is adequately treated. Instruct patients to seek medical advice if signs or symptoms suggestive of an infection occur. Exercise caution when considering use of STELARA™ in patients with a chronic infection or a history of recurrent infection.

Theoretical Risk for Vulnerability to Particular Infections

- Individuals genetically deficient in IL-12/IL-23 are particularly vulnerable to disseminated infections from mycobacteria, salmonella, and *Bacillus Calmette-Guerin* (BCG) vaccinations. Serious infections and fatal outcomes have been reported in such patients.
- It is not known whether patients with pharmacologic blockade of IL-12/IL-23 from treatment with STELARA™ will be susceptible to these types of infections. Appropriate diagnostic testing should be considered as dictated by clinical circumstances.

Pre-Treatment Evaluation of Tuberculosis (TB)

- Evaluate patients for TB infection prior to initiating treatment with STELARA™. Do not administer STELARA™ to patients with active TB. Initiate treatment of latent TB before administering STELARA™.
- Consider anti-tuberculosis therapy prior to initiation of STELARA™ in patients with a past history of latent or active tuberculosis in whom an adequate course of treatment cannot be confirmed.
- Patients receiving STELARA™ should be monitored closely for signs and symptoms of active TB during and after treatment.

Malignancies

- STELARA™ (ustekinumab) is an immunosuppressant and may increase the risk of malignancy. Malignancies were reported among subjects who received STELARA™ in clinical studies.

- The safety of STELARA™ has not been evaluated in patients who have a history of malignancy or who have a known malignancy.

Reversible Posterior Leukoencephalopathy Syndrome (RPLS)

- One case of RPLS has been reported in a STELARA™-treated subject.
- RPLS is a neurological disorder, which is not caused by demyelination or a known infectious agent. RPLS can present with headache, seizures, confusion and visual disturbances. It has been associated with preeclampsia, eclampsia, acute hypertension, cytotoxic agents and immunosuppressive therapy.
- If RPLS is suspected, discontinue STELARA™ and administer appropriate treatment.

REPORTING PATIENT ADVERSE EVENTS

It is important that you report all serious adverse events that occur in patients using STELARA™. If you have a patient who develops a serious infection or RPLS while being treated with STELARA™, or if you have a patient with cancer at any time after receiving STELARA™ therapy, it is important that you report the case even if you do not think there is a causal relationship.

The information that you can provide may inform therapy and monitoring decisions for psoriasis patients.

Reporting is easy and maintains patient confidentiality. Your patient's name or contact information is not needed. *HIPAA does not apply to* this adverse event reporting.

You can report your cases to the STELARA™ manufacturer or directly to FDA.

- Centocor Ortho Biotech Inc. at 1-800-457-6399
- MedWatch (FDA safety information and adverse event reporting program) at 1-800-332-1088 or online at www.fda.gov/medwatch/report.htm

VOLUNTARY REGISTRY INFORMATION

PSOLAR (PSOriasis Longitudinal Assessment and Registry) is a Centocor Ortho Biotech Inc.-sponsored voluntary psoriasis registry that will be available to enroll patients treated with STELARA™. Please call 1-888 PSOLAR-5 or go to www.clinicaltrials.gov for more information.

Please see the enclosed:

- STELARA™ package insert, and
- Medication Guide

Please Note: This letter does not include a comprehensive description of the serious and significant risks associated with the use of STELARA™. Please read the accompanying Full Prescribing Information and Medication Guide for a complete description of the serious and significant risk that may be associated with the use of STELARA™. You are advised to discuss the risks that may be associated with STELARA™ therapy with patients and their caregivers. We have enclosed a copy of the STELARA™ Medication Guide, which is required to be provided to patients with every filled prescription. This Medication Guide contains information that can be used to facilitate discussions about the potential risks of therapy.

Sincerely,

Peter Callegari, MD
Vice President, Medical Affairs

Enclosures

Attachment C Dear Pharmacist Letter

Centocor Ortho Biotech Inc.

IMPORTANT DRUG WARNING

Dear Pharmacist:

The purpose of this letter is to inform you of important safety information for STELARA™ (ustekinumab), a new human monoclonal antibody which has been approved by the Food and Drug Administration (FDA) for the treatment of adult patients (18 years or older) with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy. STELARA™ targets interleukin-12 (IL-12) and interleukin-23 (IL-23). FDA has determined that a Risk Evaluation and Mitigation Strategy (REMS) is necessary for STELARA™ to ensure that the benefits of the drug outweigh the potential risks of serious infections and malignancy, and reversible posterior leukoencephalopathy syndrome (RPLS).

FDA requires that a copy of the enclosed STELARA™ Medication Guide be distributed to each patient (or agent such as a caregiver) who fills a prescription for STELARA™. A copy of the STELARA™ Medication Guide will be packaged with every vial of STELARA™.

Should you require additional copies of the STELARA™ Medication Guide, you may

- Request copies from Centocor Ortho Biotech Inc. by calling the toll-free medical information line at 1-800-457-6399
- Print copies of the Medication Guide from the STELARA™ website www.stelarainfo.com
- Request copies from your drug supplier
- Photocopy the enclosed Medication Guide, after confirming that it is the most current version.
- Call the toll-free medical information line at the number above

IMPORTANT SAFETY INFORMATION FOR POTENTIAL RISKS OF SERIOUS INFECTIONS AND MALIGNANCIES, AND RPLS

Important Safety Information is listed in the Warnings and Precautions sections of the Prescribing Information for STELARA™ and Medication Guide for patients.

Infections

- STELARA™ may increase the risk of infections and reactivation of latent infections. Serious bacterial, fungal, and viral infections, some requiring hospitalization, were observed in patients receiving STELARA™. STELARA™ should not be given to patients with a clinically important active infection and should not be administered until the infection resolves or is adequately treated. Instruct patients to seek medical advice if signs or symptoms suggestive of an infection occur. Exercise caution when considering use of STELARA™ in patients with a chronic infection or a history of recurrent infection.

Theoretical Risk for Vulnerability to Particular Infections

- Individuals genetically deficient in IL-12/IL-23 are particularly vulnerable to disseminated infections from mycobacteria, salmonella, and *Bacillus Calmette-Guerin* (BCG) vaccinations. Serious infections and fatal outcomes have been reported in such patients.
- It is not known whether patients with pharmacologic blockade of IL-12/IL-23 from treatment with STELARA™ (ustekinumab) will be susceptible to these types of infections. Appropriate diagnostic testing should be considered as dictated by clinical circumstances.

Pre-Treatment Evaluation of Tuberculosis (TB)

- Evaluate patients for TB infection prior to initiating treatment with STELARA™. Do not administer STELARA™ to patients with active TB. Initiate treatment of latent TB before administering STELARA™.
- Consider anti-tuberculosis therapy prior to initiation of STELARA™ in patients with a past history of latent or active tuberculosis in whom an adequate course of treatment cannot be confirmed.
- Patients receiving STELARA™ should be monitored closely for signs and symptoms of active TB during and after treatment.

Malignancies

- STELARA™ is an immunosuppressant and may increase the risk of malignancy. Malignancies were reported among subjects who received STELARA™ in clinical studies.
- The safety of STELARA™ has not been evaluated in patients who have a history of malignancy or who have a known malignancy.

Reversible Posterior Leukoencephalopathy Syndrome (RPLS)

- One case of RPLS has been reported in a STELARA™-treated subject.
- RPLS is a neurological disorder, which is not caused by demyelination or a known infectious agent. RPLS can present with headache, seizures, confusion and visual disturbances. It has been associated with preeclampsia, eclampsia, acute hypertension, cytotoxic agents and immunosuppressive therapy.
- If RPLS is suspected, discontinue STELARA™ and administer appropriate treatment.

REPORTING PATIENT ADVERSE EVENTS

It is important that you report all serious adverse events that occur in patients using STELARA™. If you are aware of a patient who develops a serious infection or RPLS while being treated with STELARA™, or if you are aware of a patient with cancer at any time after receiving STELARA™ therapy, it is important that you report the case even if you do not think there is a causal relationship. The information that you can provide may inform therapy and monitoring decisions.

Reporting is easy and maintains patient confidentiality. The patient's name or contact information is not needed. *HIPAA does not apply to* this adverse event reporting.

You can report cases to the STELARA™ manufacturer or directly to FDA.

- Centocor Ortho Biotech Inc. at 1-800-457-6399
- MedWatch (FDA safety information and adverse event reporting program) at 1-800-332-1088 or online at www.fda.gov/medwatch/report.htm

Please Note: This letter does not include a comprehensive description of the serious and significant risks that may be associated with the use of STELARA™. Please read the accompanying Full Prescribing Information and Medication Guide for a complete description of the serious and significant risk that may be associated with the use of STELARA™.

Sincerely,

Peter Callegari, MD
Vice President, Medical Affairs

Enclosures

Attachment D Journal Information Piece for Dermatologists

Important Information for Dermatologists about the Potential Risks of Serious Infections and Malignancy, and RPLS with STELARA™ for Psoriasis Therapy

STELARA™ (ustekinumab) is a new human monoclonal antibody that is indicated for the treatment of adult patients (18 years or older) with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy.

STELARA™ targets interleukin-12 (IL-12) and interleukin-23 (IL-23). Based on data from rodent models and humans genetically deficient for components of IL-12 and IL-23 pathways, there is a theoretical concern that **blockade of IL-12 and IL-23 may increase the risk for serious infections, including mycobacterial and recurrent salmonella infections.** In addition, data from rodent models suggest there is a theoretical concern that **blockade of IL-12 and IL-23 may increase the risk for malignancies.**

One case of Reversible Posterior Leukoencephalopathy Syndrome (RPLS) has been reported in a STELARA™-treated patient in clinical trials. RPLS is a neurological disorder which is not caused by demyelination or a known infectious agent, and can present with headache, seizures, confusion and visual disturbances.

If you have a patient that develops a serious infection or RPLS while being treated with STELARA™, or if you have a patient with cancer at any time after receiving STELARA™ therapy, it is important that you report the case even if you do not think there is a causal relationship.

The information that you, as a STELARA™ prescribing dermatologist, can provide may inform therapy and monitoring decisions for psoriasis patients.

Reporting is easy and maintains patient confidentiality. Your patient's name or contact information is not needed. *HIPAA does not apply to* this adverse event reporting.

You can report your cases to the STELARA™ manufacturer or directly to FDA.

- Centocor Ortho Biotech Inc. at 1-800-457-6399
- MedWatch (FDA safety information and adverse event reporting program) at 1-800-332-1088 or online at www.fda.gov/medwatch/report.htm

PSOLAR (Psoriasis Longitudinal Assessment and Registry): is a **voluntary, disease-specific registry,** developed by Centocor Ortho Biotech Inc., that collects information from psoriasis patients and their treating physicians. Since this registry will continue for 10 years, it will help us better understand the risk of long-latency serious events, such as malignancies, that can occur after exposure to a product such as STELARA™.

For more information on PSOLAR and how to include patients in this voluntary registry, call 1-888-PSOLAR5 (1-888-776-5275) or access www.clinicaltrials.gov and search for PSOLAR.

Centocor Ortho Biotech Inc.

Attachment E Journal Information Piece for Oncologists

Important Information for Oncologists About Potential Malignancy Risk With STELARA™ for Psoriasis Therapy

STELARA™ (ustekinumab) is a new human monoclonal antibody that is indicated for the treatment of adult patients (18 years or older) with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy.

STELARA™ targets interleukin-12 (IL-12) and interleukin-23 (IL-23). Based on data from rodent models, there is a theoretical concern that **blockade of IL-12 and IL-23 may increase the risk for malignancies.**

If you are consulted to see a patient with cancer at any time after receiving STELARA™ therapy, it is important that you report the case even if you do not think there is a causal relationship.

The information that you, as an oncologist, can provide may inform therapy and monitoring decisions for psoriasis patients.

Reporting is easy and maintains patient confidentiality. Your patient's name or contact information is not needed. *HIPAA does not apply to* this adverse event reporting.

You can report your cases to the STELARA™ manufacturer or directly to FDA.

- Centocor Ortho Biotech Inc. at 1-800-457-6399
- MedWatch (FDA safety information and adverse event reporting program) at 1-800-332-1088 or online at www.fda.gov/medwatch/report.htm

Centocor Ortho Biotech Inc.

Attachment F Journal Information Piece for Rheumatologists

Important Information for Rheumatologists about the Potential Risks of Serious Infections and Malignancy, and RPLS with STELARA™ for Psoriasis Therapy

STELARA™ (ustekinumab) is a new human monoclonal antibody that is indicated for the treatment of adult patients (18 years or older) with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy.

STELARA™ targets interleukin-12 (IL-12) and interleukin-23 (IL-23). Based on data from rodent models and humans genetically deficient for components of IL-12 and IL-23 pathways, there is a theoretical concern that **blockade of IL-12 and IL-23 may increase the risk for serious infections, including mycobacterial and recurrent salmonella infections.** In addition, data from rodent models suggest there is a theoretical concern that **blockade of IL-12 and IL-23 may increase the risk for malignancies.**

One case of Reversible Posterior Leukoencephalopathy Syndrome (RPLS) has been reported in a STELARA™-treated patient in clinical trials. RPLS is a neurological disorder which is not caused by demyelination or a known infectious agent, and can present with headache, seizures, confusion and visual disturbances.

If you have a patient that develops a serious infection or RPLS while being treated with STELARA™, or if you have a patient with cancer at any time after receiving STELARA™ therapy, it is important that you report the case even if you do not think there is a causal relationship.

The information that you, as a rheumatologist that may co-manage patients receiving STELARA™ therapy, can provide may inform therapy and monitoring decisions for psoriasis patients.

Reporting is easy and maintains patient confidentiality. Your patient's name or contact information is not needed. *HIPAA does not apply to* this adverse event reporting.

You can report your cases to the STELARA™ manufacturer or directly to FDA.

- Centocor Ortho Biotech Inc. at 1-800-457-6399
- MedWatch (FDA safety information and adverse event reporting program) at 1-800-332-1088 or online at www.fda.gov/medwatch/report.htm

Centocor Ortho Biotech Inc.

Attachment G Journal Information Piece for Infectious Disease Specialists

Important Information for Infectious Disease Specialists About Potential Serious Infection Risk With STELARA™ for Psoriasis Therapy

STELARA™ (ustekinumab) is a new human monoclonal antibody that is indicated for the treatment of adult patients (18 years or older) with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy.

STELARA™ targets interleukin-12 (IL-12) and interleukin-23 (IL-23). Based on data from rodent models and humans genetically deficient for components of IL-12 and IL-23 pathways, there is a theoretical concern that **blockade of IL-12 and IL-23 may increase the risk for serious infections, including mycobacterial and recurrent salmonella infections.**

If you are consulted to see a patient that develops a serious infection while being treated with STELARA™, it is important that you report the case even if you do not think there is a causal relationship.

The information that you, as an infectious disease specialist, can provide may inform therapy and monitoring decisions for psoriasis patients.

Reporting is easy and maintains patient confidentiality. Your patient's name or contact information is not needed. *HIPAA does not apply to* this adverse event reporting.

You can report your cases to the STELARA™ manufacturer or directly to FDA.

- Centocor Ortho Biotech Inc. at 1-800-457-6399
- MedWatch (FDA safety information and adverse event reporting program) at 1-800-332-1088 or online at www.fda.gov/medwatch/report.htm

Centocor Ortho Biotech Inc.

Attachment H Journal Information Piece for Gastroenterologists

Important Information for Gastroenterologists About Potential Serious Infection Risk With STELARA™ for Psoriasis Therapy

STELARA™ (ustekinumab) is a new human monoclonal antibody that is indicated for the treatment of adult patients (18 years or older) with moderate to severe plaque psoriasis who are candidates for phototherapy or systemic therapy.

STELARA™ targets interleukin-12 (IL-12) and interleukin-23 (IL-23). Based on data from rodent models and humans genetically deficient for components of IL-12 and IL-23 pathways, there is a theoretical concern that **blockade of IL-12 and IL-23 may increase the risk for serious infections, including mycobacterial and recurrent salmonella infections.**

If you are consulted to see a patient that develops a serious infection while being treated with STELARA™, it is important that you report the case even if you do not think there is a causal relationship.

The information that you, as a gastroenterologist, can provide may inform therapy and monitoring decisions for psoriasis patients.

Reporting is easy and maintains patient confidentiality. Your patient's name or contact information is not needed. *HIPAA does not apply to* this adverse event reporting.

You can report your cases to the STELARA™ manufacturer or directly to FDA.

- Centocor Ortho Biotech Inc. at 1-800-457-6399
- MedWatch (FDA safety information and adverse event reporting program) at 1-800-332-1088 or online at www.fda.gov/medwatch/report.htm

Centocor Ortho Biotech Inc.

Exhibit 2

Copy of U.S. Patent No. 6,902,734

US006902734B2

(12) **United States Patent**
Giles-Komar et al.

(10) **Patent No.: US 6,902,734 B2**
(45) **Date of Patent: Jun. 7, 2005**

(54) **ANTI-IL-12 ANTIBODIES AND COMPOSITIONS THEREOF**

(75) Inventors: **Jill Giles-Komar**, Downingtown, PA (US); **David M. Knight**, Berwyn, PA (US); **David Peritt**, Bala Cynwyd, PA (US); **Bernard Scallon**, Collegeville, PA (US); **David Shealy**, Downingtown, PA (US)

(73) Assignee: **Centocor, Inc.**, Malvern, PA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 360 days.

(21) Appl. No.: **09/920,262**

(22) Filed: **Aug. 1, 2001**

(65) **Prior Publication Data**

US 2003/0124123 A1 Jul. 3, 2003

Related U.S. Application Data

(60) Provisional application No. 60/236,827, filed on Sep. 29, 2000, and provisional application No. 60/223,358, filed on Aug. 7, 2000.

(51) **Int. Cl.**[7] **C07K 16/24**; A61K 39/395

(52) **U.S. Cl.** **424/145.1**; 424/130.1; 424/139.1; 530/387.1; 530/387.9; 530/388.23; 530/389.2

(58) **Field of Search** 530/387.1, 387.9, 530/388.23, 389.2; 424/130.1, 139.1, 145.1

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R.W. Carter. et. al. (Production and Characterization of Monoclonal Antibodies to Human Interleukin–12) Hybriidona 16: 363–369 (1997).

(Continued)

Primary Examiner—Prema Mertz
(74) *Attorney, Agent, or Firm*—Eric A. Dichter; Guy Kevin Townsend

(57) **ABSTRACT**

The present invention relates to at least one anti-IL-12 antibody, including isolated nucleic acids that encode at least one anti-IL-12 antibody, IL-12, vectors, host cells, transgenic animals or plants, and methods of making and using thereof, including therapeutic compositions, methods and devices.

4 Claims, 12 Drawing Sheets

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PCT International Search Report PCT/US01/24720 dated Jul. 30, 2002.

FIG. 1A

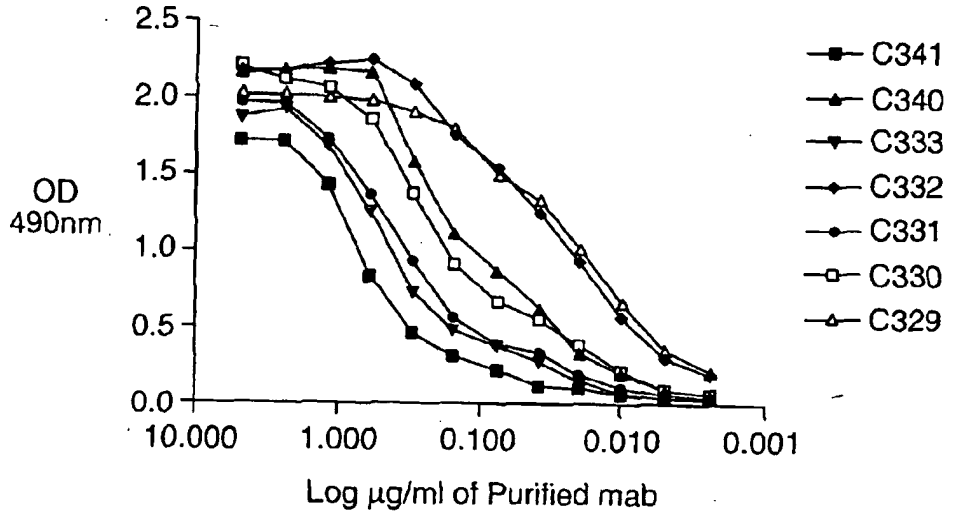
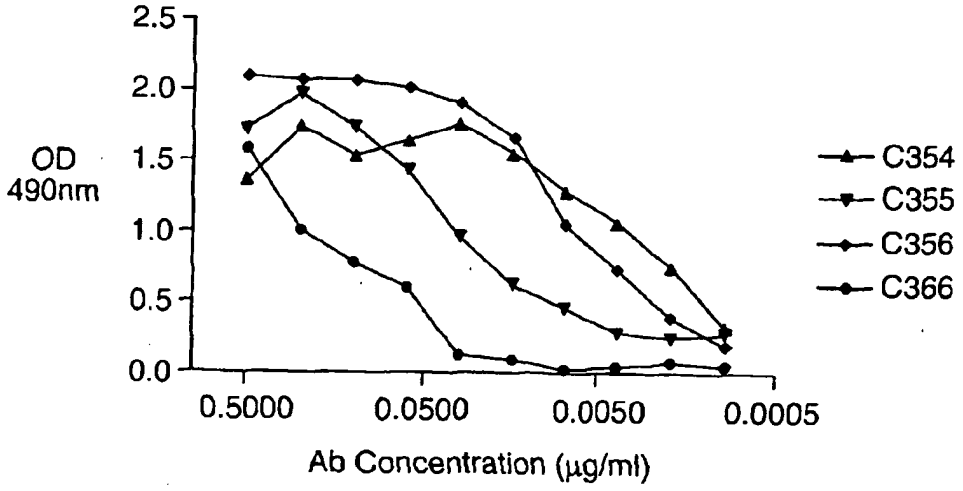

2.5
2.0
1.5
1.0
0.5
0.0
OD
490nm
10.000
1.000
0.100
0.010
0.001
Log µg/ml of Purified mab
C341
C340
C333
C332
C331
C330
C329

FIG. 1B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2.0
1.5
1.0
0.5
0.0
OD
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0.0500
0.0050
0.0005
Ab Concentration (µg/ml)
C354
C355
C356
C366

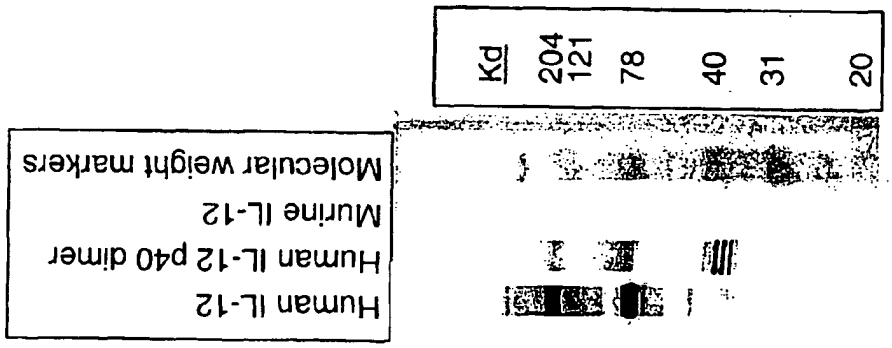

Kd
204
121
78
40
31
20
Human IL-12
Human IL-12 p40 dimer
Murine IL-12
Molecular weight markers

FIG. 2B

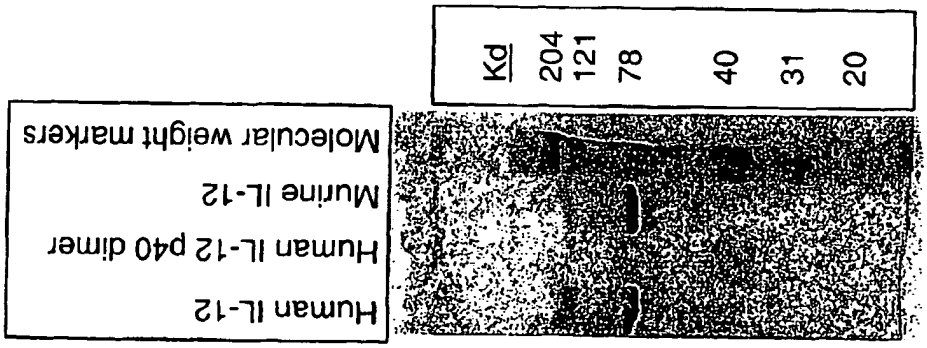

Kd
204
121
78
40
31
20
Human IL-12
Human IL-12 p40 dimer
Murine IL-12
Molecular weight markers

FIG. 2A

FIG. 3

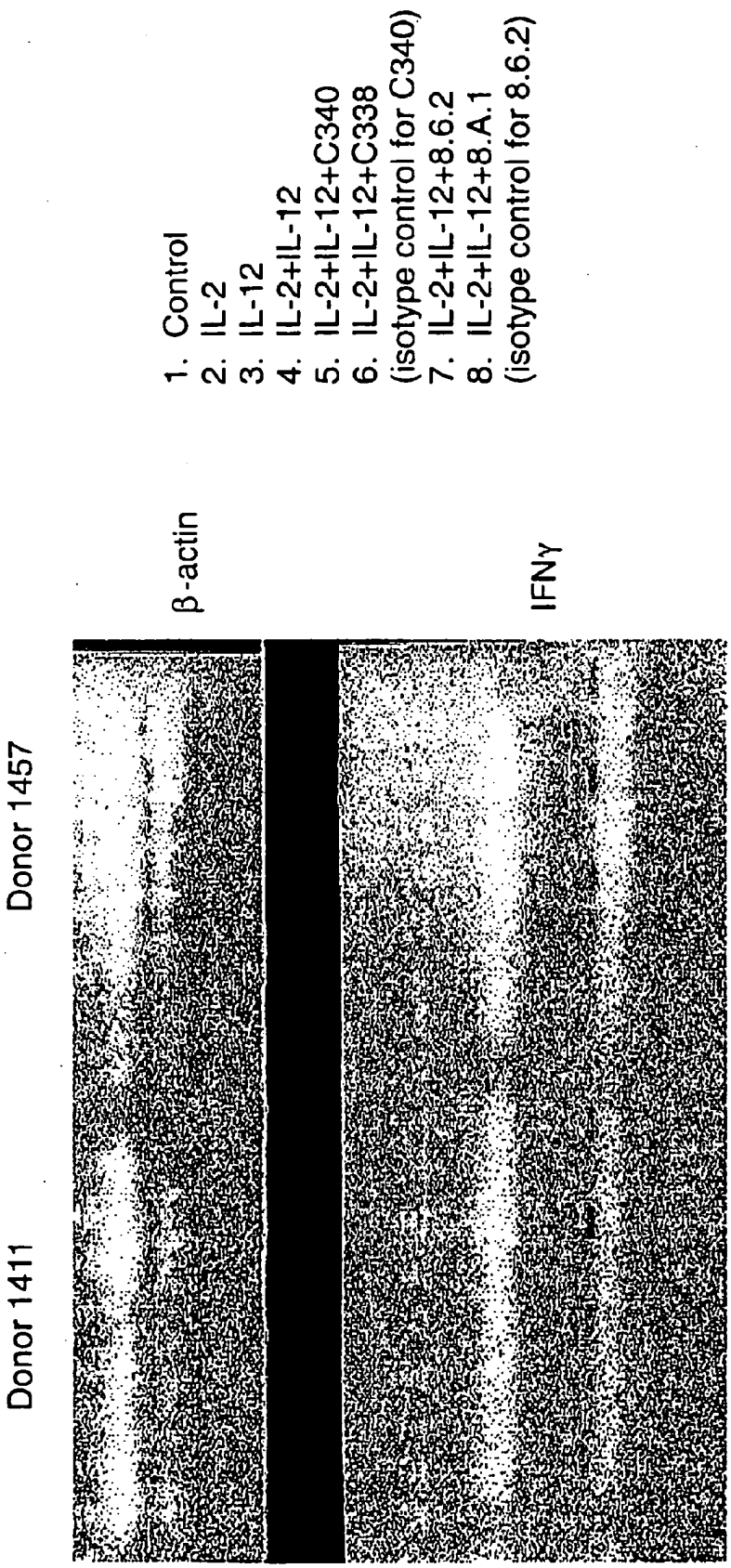

Donor 1411
Donor 1457
β-actin
IFNγ
1. Control
2. IL-2
3. IL-12
4. IL-2+IL-12
5. IL-2+IL-12+C340
6. IL-2+IL-12+C338
(isotype control for C340)
7. IL-2+IL-12+8.6.2
8. IL-2+IL-12+8.A.1
(isotype control for 8.6.2)

FIG. 4

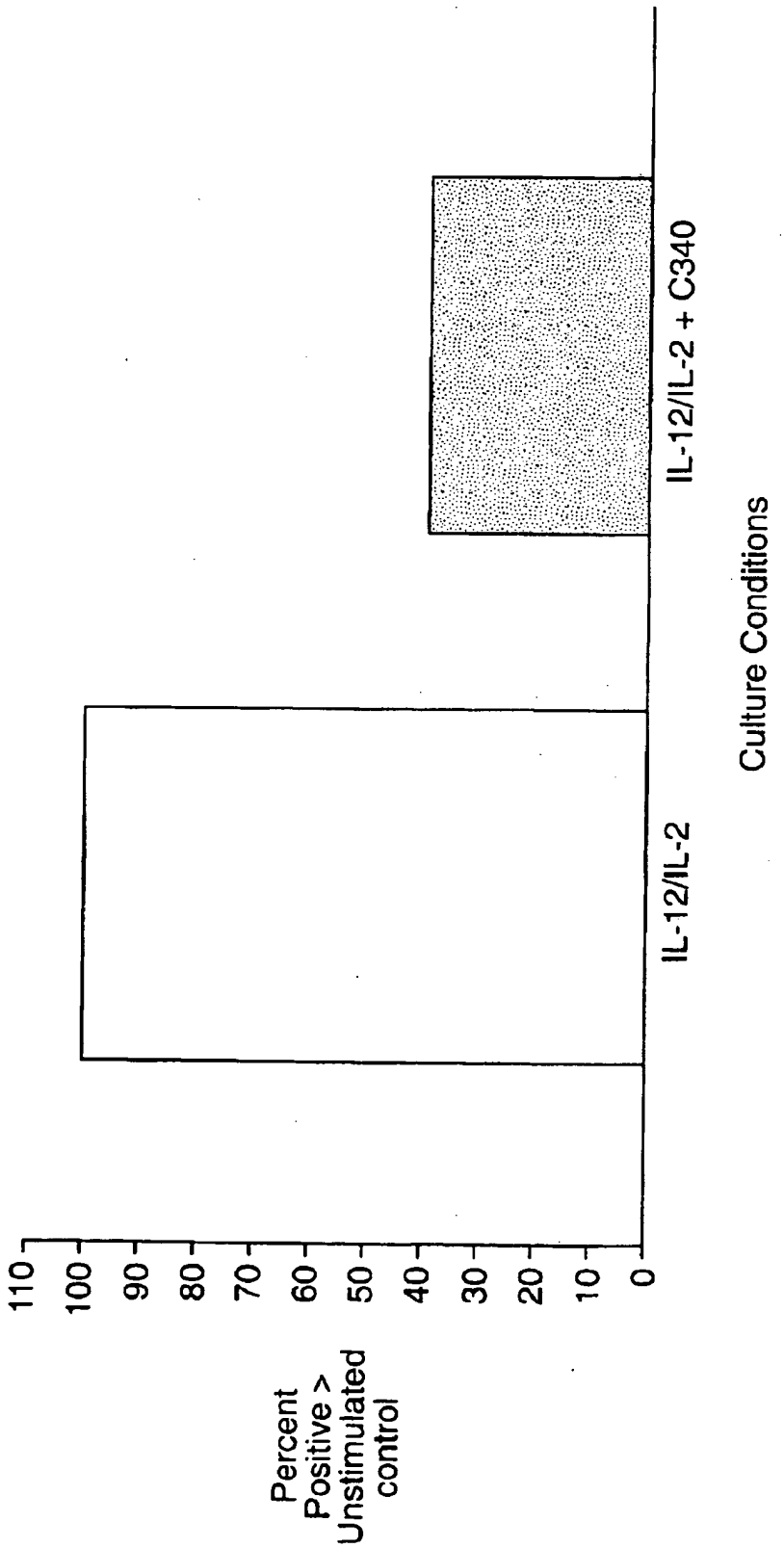

Percent Positive > Unstimulated control
110
100
90
80
70
60
50
40
30
20
10
0
IL-12/IL-2
IL-12/IL-2 + C340
Culture Conditions

FIG. 5

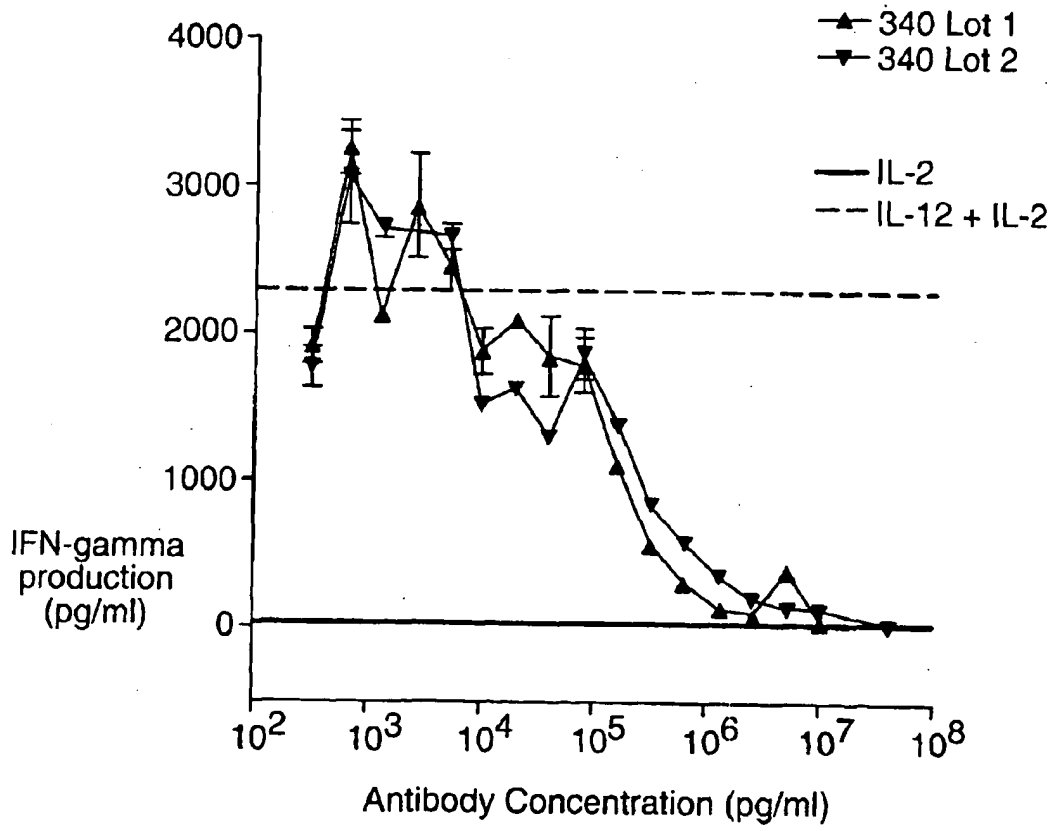

340 Lot 1
340 Lot 2
IL-2
IL-12 + IL-2
4000
3000
2000
1000
0
IFN-gamma production (pg/ml)
10^2
10^3
10^4
10^5
10^6
10^7
10^8
Antibody Concentration (pg/ml)

FIG. 6

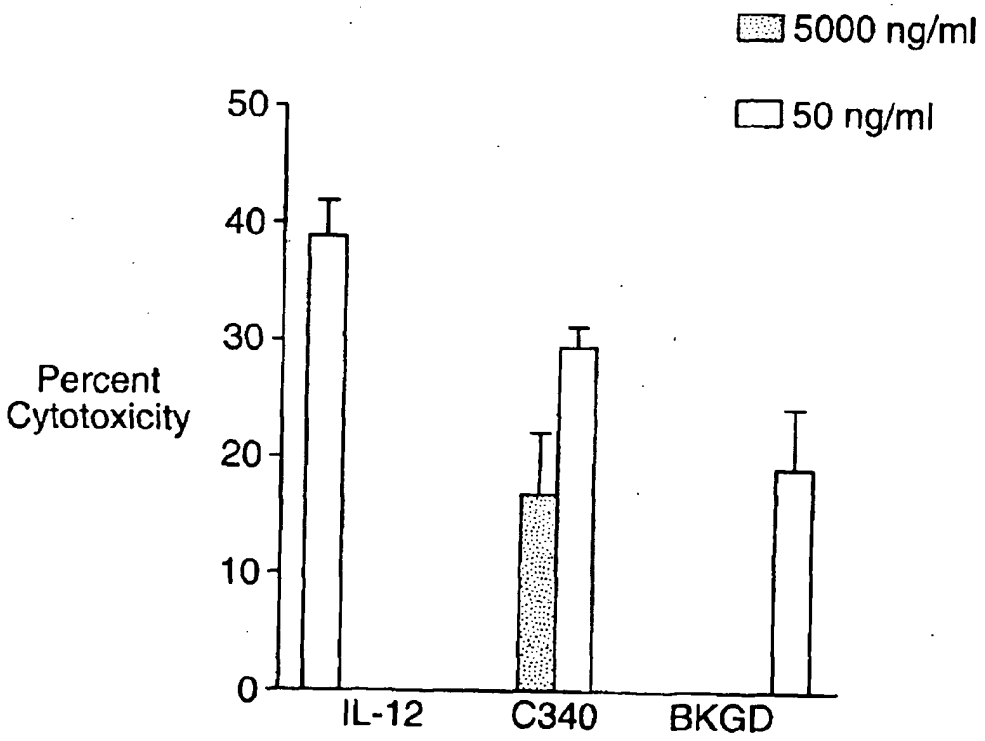

5000 ng/ml
50 ng/ml
Percent Cytotoxicity
50
40
30
20
10
0
IL-12
C340
BKGD

FIG. 7A

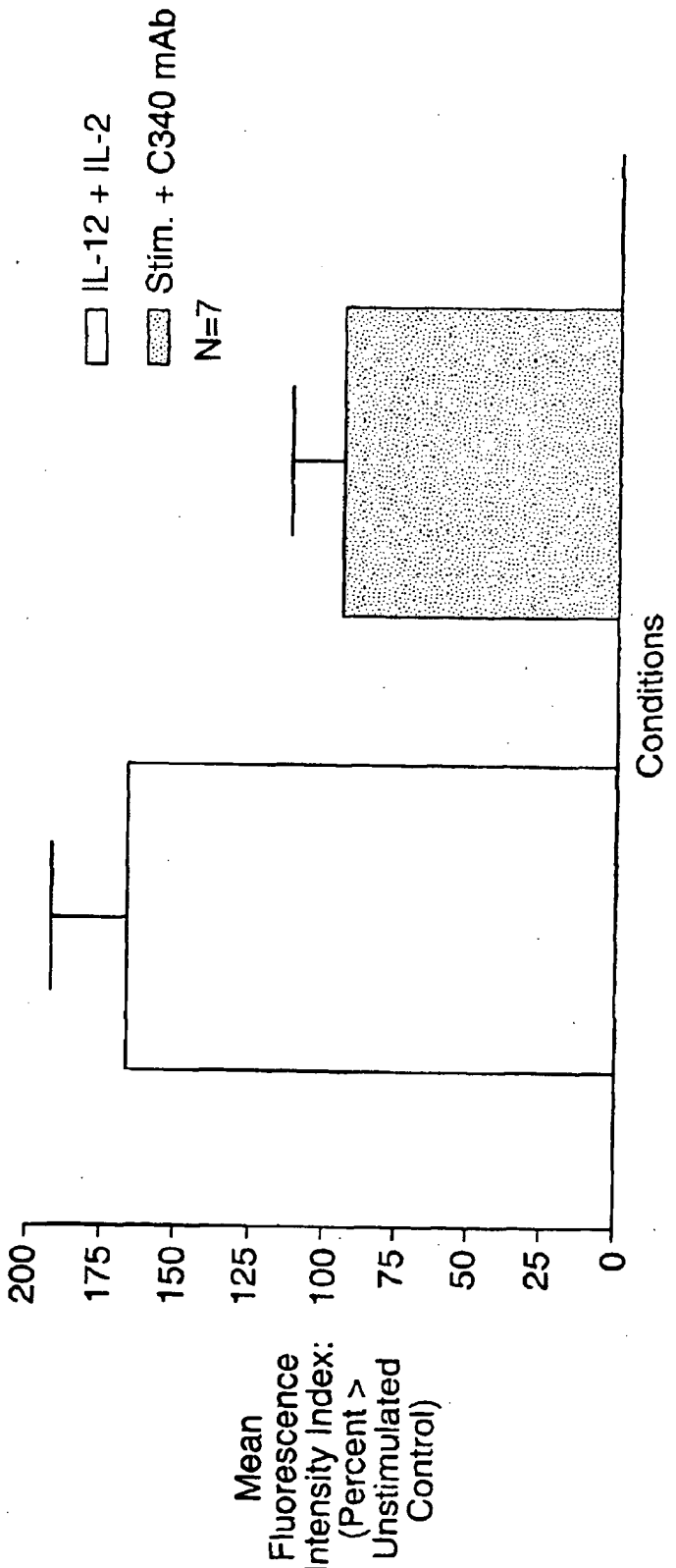

IL-12 + IL-2
Stim. + C340 mAb
N=7
Conditions
Mean Fluorescence Intensity Index: (Percent > Unstimulated Control)
200
175
150
125
100
75
50
25
0

FIG. 7B

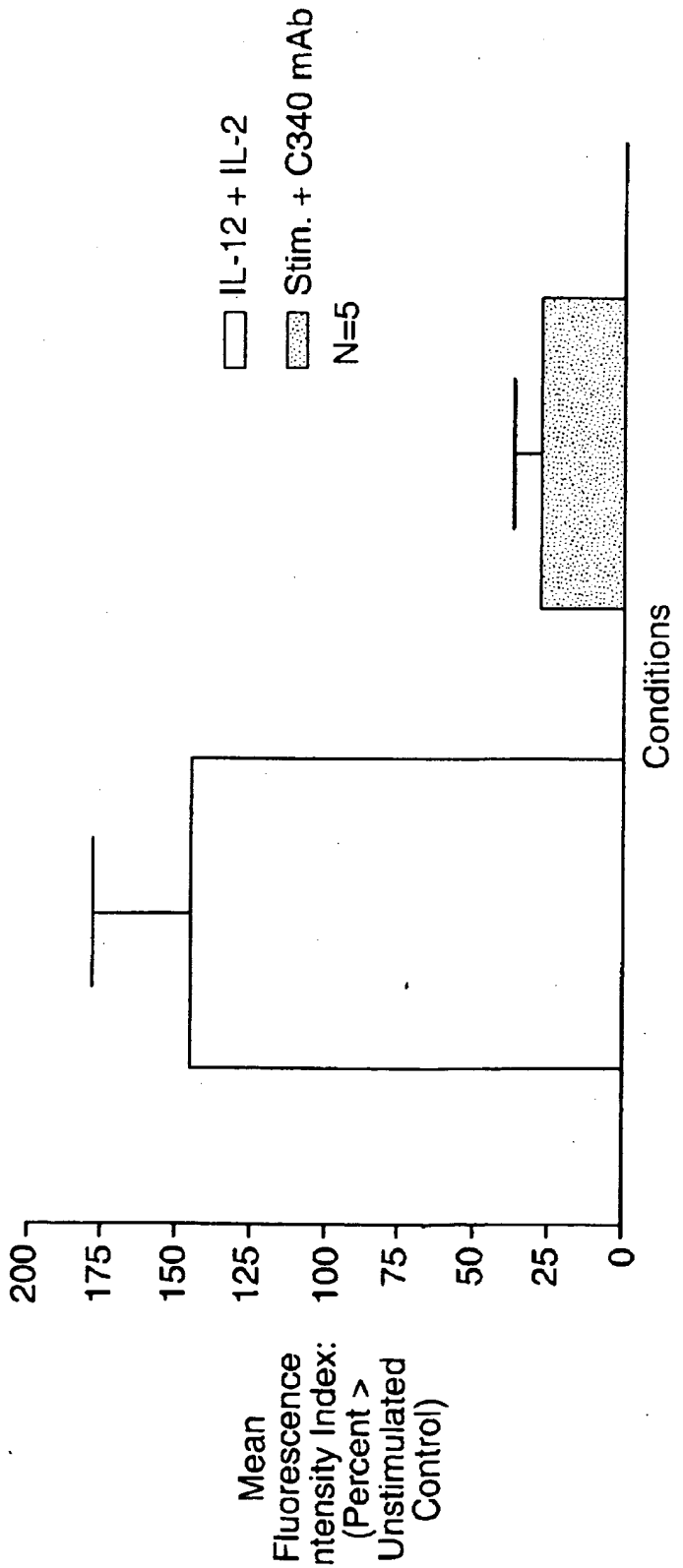

IL-12 + IL-2
Stim. + C340 mAb
N=5
Mean Fluorescence Intensity Index: (Percent > Unstimulated Control)
200
175
150
125
100
75
50
25
0
Conditions

FIG. 8

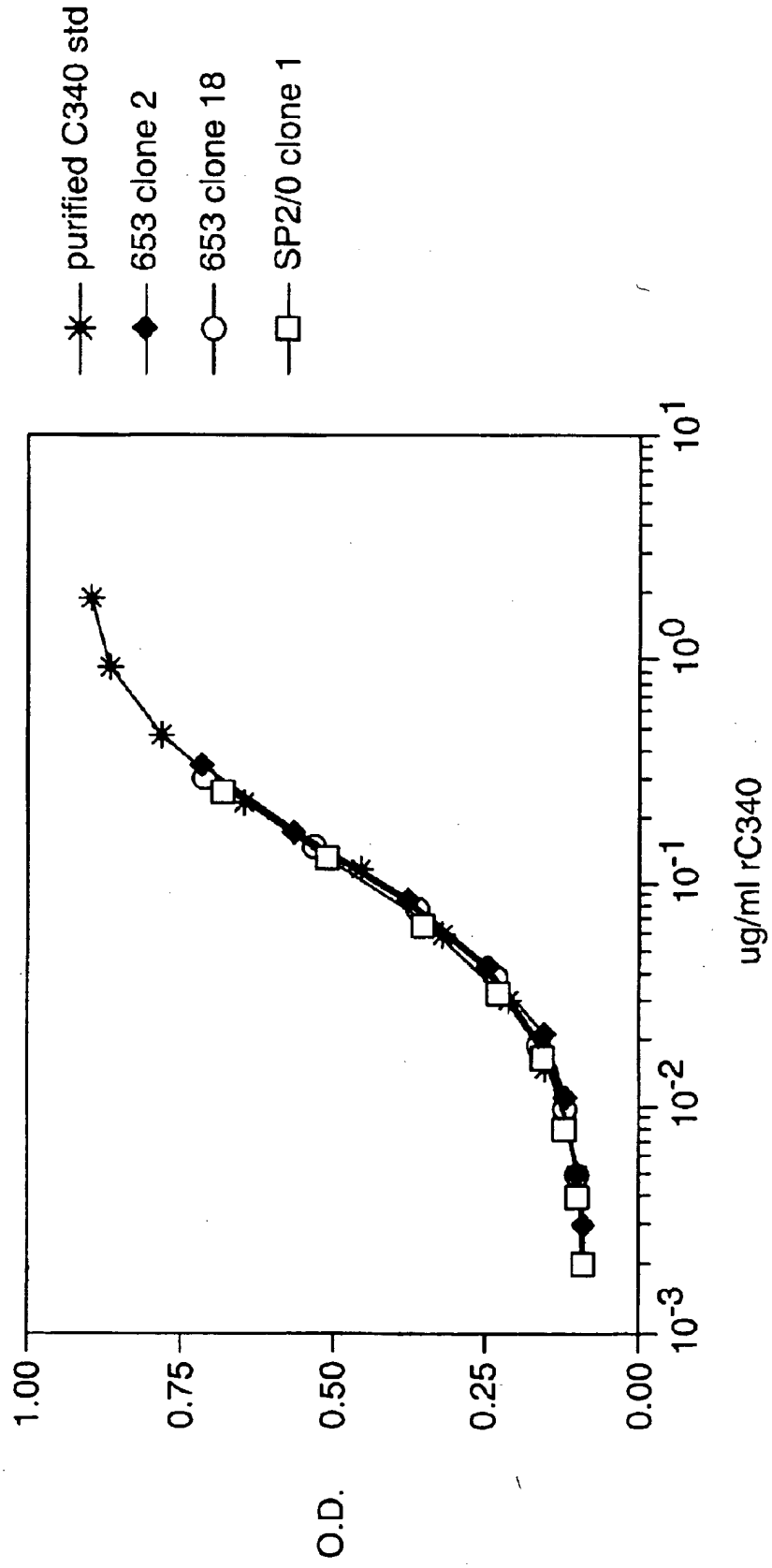
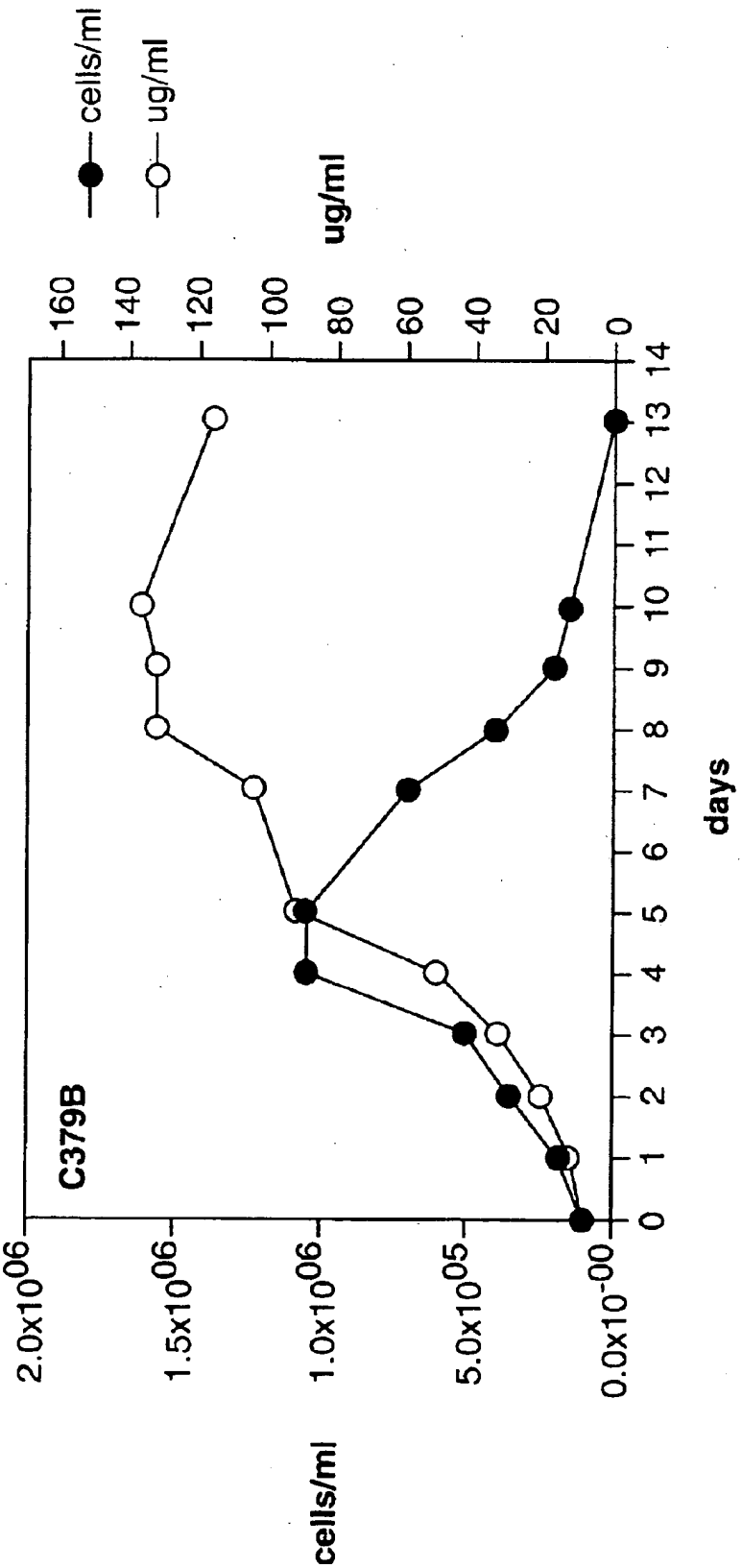

purified C340 std
653 clone 2
653 clone 18
SP2/0 clone 1
O.D.
1.00
0.75
0.50
0.25
0.00
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
ug/ml rC340

FIG. 9A


cells/ml
ug/ml
C379B
cells/ml
ug/ml
days
2.0x10^06
1.5x10^06
1.0x10^06
5.0x10^05
0.0x10^-00
160
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

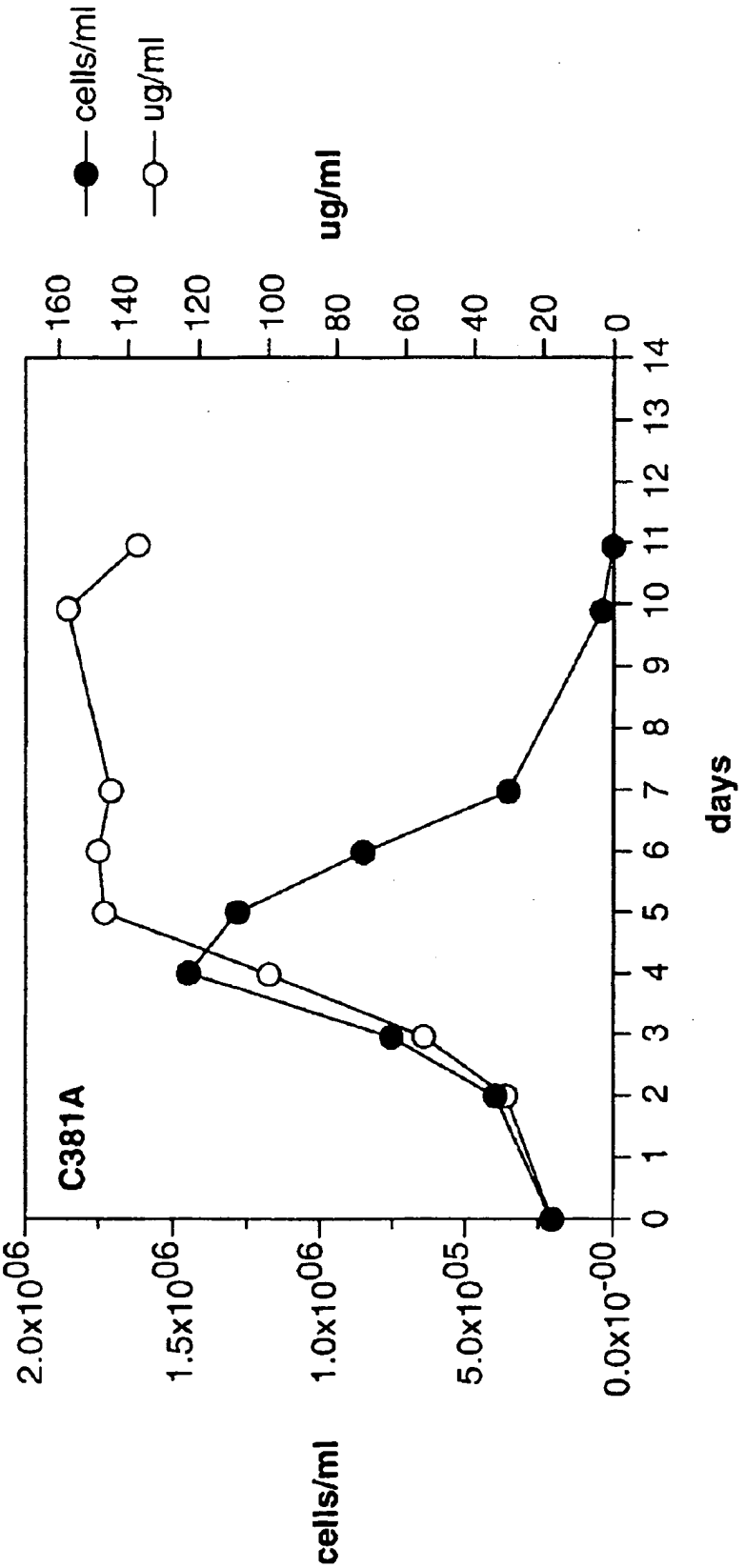

cells/ml
ug/ml
C381A
cells/ml
ug/ml
days
2.0x10^06
1.5x10^06
1.0x10^06
5.0x10^05
0.0x10^-00
160
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIG. 9B

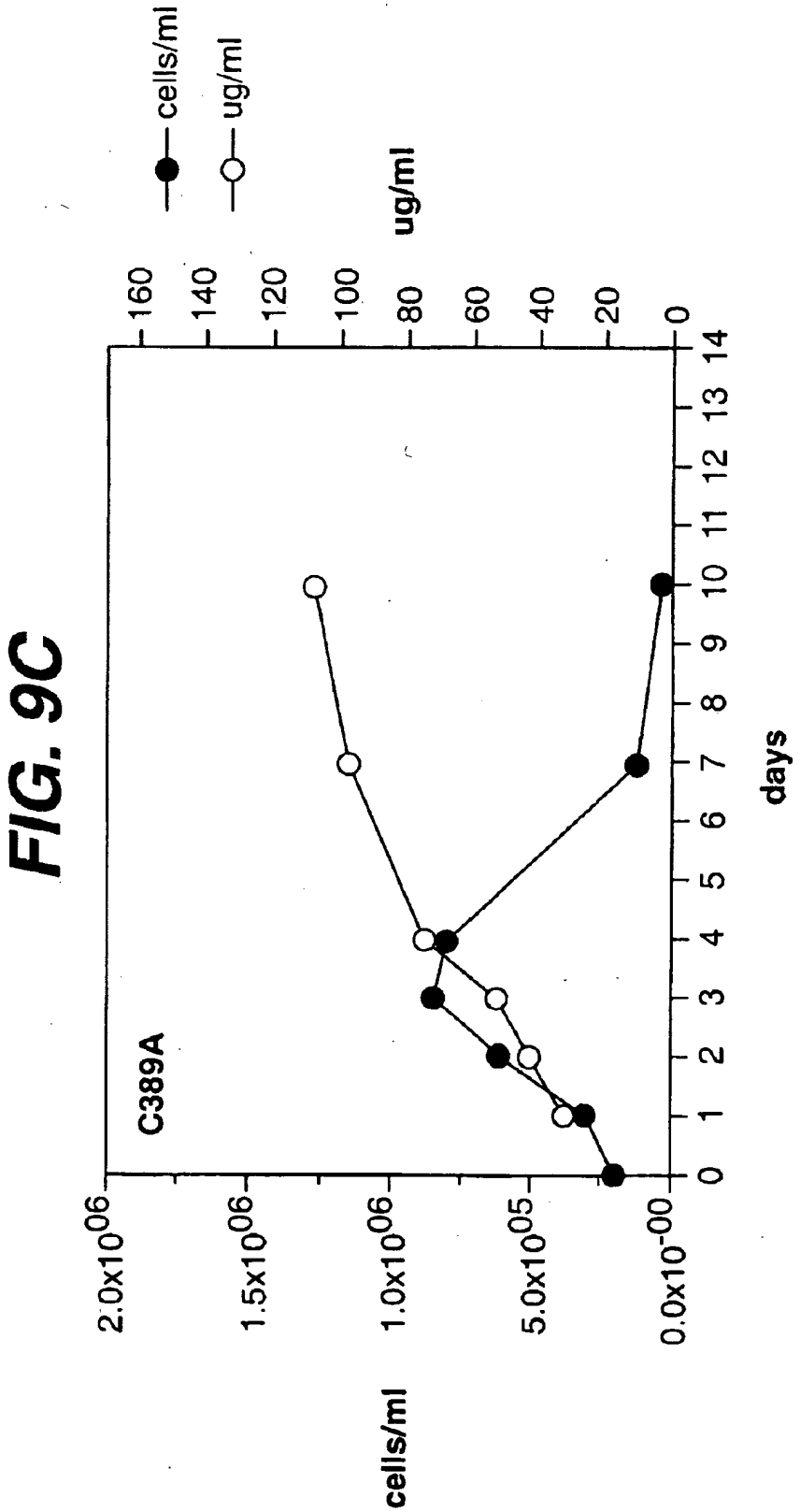

cells/ml
ug/ml
C389A
cells/ml
ug/ml
days
2.0x10^06
1.5x10^06
1.0x10^06
5.0x10^05
0.0x10^-00
160
140
120
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

FIG. 9C

ANTI-IL-12 ANTIBODIES AND COMPOSITIONS THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application is based in part on, and claims priority to, U.S. Provisional Application Nos. 60/223,358 filed Aug. 7, 2000 and 60/236,827 filed Sep. 29, 2000, each of which is entirely incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to antibodies, including specified portions or variants, specific for at least one Interleukin-12 (IL-12) protein or fragment thereof, as well as nucleic acids encoding such anti-IL-12 antibodies, complementary nucleic acids, vectors, host cells, and methods of making and using thereof, including therapeutic formulations, administration and devices.

2. Related Art

Interleukin-12 (IL-12) is a heterodimeric cytokine consisting of glycosylated polypeptide chains of 35 and 40 kD which are disulfide bonded. The cytokine is synthesized and secreted by antigen presenting cells including dendritic cells, monocytes, macrophages, B cells, Langerhans cells and keratinocytes as well as natural killer (NK) cells. IL-12 mediates a variety of biological processes and has been referred to as NK cell stimulatory factor (NKSF), T-cell stimulating factor, cytotoxic T-lymphocyte maturation factor and EBV-transformed B-cell line factor (Curfs, J. H. A. J., et al., Clinical Microbiology Reviews, 10:742–780 (1997)).

Interleukin-12 can bind to the IL-12 receptor expressed on the plasma membrane of cells (e.g., T cells, NK cell), thereby altering (e.g., initiating, preventing) biological processes. For example, the binding of IL-12 to the IL-12 receptor can stimulate the proliferation of pre-activated T cells and NK cells, enhance the cytolytic activity of cytotoxic T cells (CTL), NK cells and LAK (lymphokine activated killer) cells, induce production of gamma interferon (IFN GAMMA) by T cells and NK cells and induce differentiation of naive Th0 cells into Th1 cells that produce IFN GAMMA and IL-2 (Trinchieri, G., Annual Review of Immunology, 13:251–276 (1995)). In particular, IL-12 is vital for the generation of cytolytic cells (e.g., NK, CTL) and for mounting a cellular immune response (e.g., a Th1 cell mediated immune response). Thus, IL-12 is critically important in the generation and regulation of both protective immunity (e.g., eradication of infections) and pathological immune responses (e.g., autoimmunity) (Hendrzak, J. A. and Brunda, M. J., Laboratory Investigation, 72:619–637 (1995)). Accordingly, an immune response (e.g., protective or pathogenic) can be enhanced, suppressed or prevented by manipulation of the biological activity of IL-12 in vivo, for example, by means of an antibody.

Non-human mammalian, chimeric, polyclonal (e.g., antisera) and/or monoclonal antibodies (Mabs) and fragments (e.g., proteolytic digestion or fusion protein products thereof) are potential therapeutic agents that are being investigated in some cases to attempt to treat certain diseases. However, such antibodies or fragments can elicit an immune response when administered to humans. Such an immune response can result in an immune complex-mediated clearance of the antibodies or fragments from the circulation, and make repeated administration unsuitable for therapy, thereby reducing the therapeutic benefit to the patient and limiting the readministration of the antibody or fragment. For example, repeated administration of antibodies or fragments comprising non-human portions can lead to serum sickness and/or anaphalaxis. In order to avoid these and other problems, a number of approaches have been taken to reduce the immunogenicity of such antibodies and portions thereof, including chimerization and humanization, as well known in the art. These and other approaches, however, still can result in antibodies or fragments having some immunogenicity, low affinity, low avidity, or with problems in cell culture, scale up, production, and/or low yields. Thus, such antibodies or fragments can be less than ideally suited for manufacture or use as therapeutic proteins.

Accordingly, there is a need to provide anti-IL-12 antibodies or fragments that overcome one more of these problems, as well as improvements over known antibodies or fragments thereof.

SUMMARY OF THE INVENTION

The present invention provides isolated human, primate, rodent, mammalian, chimeric, humanized and/or CDR-grafted anti-IL-12 antibodies, immunoglobulins, cleavage products and other specified portions and variants thereof, as well as anti-IL-12 antibody compositions, encoding or complementary nucleic acids, vectors, host cells, compositions, formulations, devices, transgenic animals, transgenic plants, and methods of making and using thereof, as described and enabled herein, in combination with what is known in the art.

The present invention also provides at least one isolated anti-IL-12 antibody as described herein. An antibody according to the present invention includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to at least one complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into an antibody of the present invention. An antibody of the invention can include or be derived from any mammal, such as but not limited to a human, a mouse, a rabbit, a rat, a rodent, a primate, or any combination thereof, and the like.

The present invention provides, in one aspect, isolated nucleic acid molecules comprising, complementary, or hybridizing to, a polynucleotide encoding specific anti-IL-12 antibodies, comprising at least one specified sequence, domain, portion or variant thereof. The present invention further provides recombinant vectors comprising said anti-IL-12 antibody nucleic acid molecules, host cells containing such nucleic acids and/or recombinant vectors, as well as methods of making and/or using such antibody nucleic acids, vectors and/or host cells.

At least one antibody of the invention binds at least one specified epitope specific to at least one IL-12 protein, subunit, fragment, portion or any combination thereof. The at least one epitope can comprise at least one antibody binding region that comprises at least one portion of said protein, which epitope is preferably comprised of at least 1–5 amino acids of at least one portion thereof, such as but not limited to, at least one functional, extracellular, soluble, hydrophillic, external or cytoplasmic domain of said protein, or any portion thereof.

The at least one antibody can optionally comprise at least one specified portion of at least one complementarity determining region (CDR) (e.g., CDR1, CDR2 or CDR3 of the

heavy or light chain variable region) and/or at least one constant or variable framework region or any portion thereof. The at least one antibody amino acid sequence can further optionally comprise at least one specified substitution, insertion or deletion as described herein or as known in the art.

The present invention also provides at least one isolated anti-IL-12 antibody as described herein, wherein the antibody has at least one activity, such as, but not limited to: (i) inhibition of IL-12 induced IFN-gamma secretion; (ii) inhibition of LAK cell cytotoxicity; (iii) inhibition of IFN gamma mRNA transription; (iv) inhibition of intracellular IFN gamma CD3+ cells; and/or (v) CD95 expression. See, e.g., Chan, et al., (1992). J. Immunol. 148(1): 92–98; Chan, et al., (1991). J. Exp. Med. 173(4): 869–79; Chehimi, et al., (1992) J. Exp. Med. 175(3): 789–96; Medvedev, et al., (1997) Cytokine 9(6): 394–404. A(n) anti-IL-12 antibody can thus be screened for a corresponding activity according to known methods, such as but not limited to at least one biological activity towards a IL-12 protein.

The present invention further provides at least one IL-12 anti-idiotype antibody to at least one IL-12 antibody of the present invention. The anti-idiotype antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to at least one complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, that can be incorporated into an antibody of the present invention. An antibody of the invention can include or be derived from any mammal, such as but not limited to a human, a mouse, a rabbit, a rat, a rodent, a primate, and the like.

The present invention provides, in one aspect, isolated nucleic acid molecules comprising, complementary, or hybridizing to, a polynucleotide encoding at least one IL-12 anti-idiotype antibody, comprising at least one specified sequence, domain, portion or variant thereof. The present invention further provides recombinant vectors comprising said IL-12 anti-idiotype antibody encoding nucleic acid molecules, host cells containing such nucleic acids and/or recombinant vectors, as well as methods of making and/or using such anti-idiotype antibody nucleic acids, vectors and/or host cells.

The present invention also provides at least one method for expressing at least one anti-IL-12 antibody, or IL-12 anti-idiotype antibody, in a host cell, comprising culturing a host cell as described herein under conditions wherein at least one anti-IL-12 antibody is expressed in detectable and/or recoverable amounts.

The present invention also provides at least one composition comprising (a) an isolated anti-IL-12 antibody encoding nucleic acid and/or antibody as described herein; and (b) a suitable carrier or diluent. The carrier or diluent can optionally be pharmaceutically acceptable, according to known carriers or diluents. The composition can optionally further comprise at least one further compound, protein or composition.

The present invention further provides at least one anti-IL-12 antibody method or composition, for administering a therapeutically effective amount to modulate or treat at least one IL-12 related condition in a cell, tissue, organ, animal or patient and/or, prior to, subsequent to, or during a related condition, as described herein.

The present invention also provides at least one composition, device and/or method of delivery of a therapeutically or prophylactically effective amount of at least one anti-IL-12 antibody, according to the present invention.

The present invention further provides at least one anti-IL-12 antibody method or composition, for diagnosing at least one IL-12 related condition in a cell, tissue, organ, animal or patient and/or, prior to, subsequent to, or during a related condition, as described herein.

The present invention also provides at least one composition, device and/or method of delivery for diagnosing of at least one anti-IL-12 antibody, according to the present invention.

DESCRIPTION OF THE FIGURES

FIGS. **1A** and **1B** are graphs showing concentration-dependent binding of human anti-IL-12 mAbs to immobilized human IL-12. Anti-IL-12 antibodies were serially diluted in 1% BSA/PBS and incubated on rhIL-12 coated plates for 1 hour at 37° C. Plates were washed twice with 0.02% Tween 20 (polyoxyethylene (20) sorbitan monolaurate), 0.15M saline and then probed with horse radish peroxidase (HRP) labeled goat anti-human IgG kappa specific antibody for 1 hour at room temperature. Plates were again washed, developed with o-phenylenediamine (OPD) substrate and the optical density (OD) of each well was measured at 490 nm.

FIG. **2**: Lanes from left to right in Figures A and B contain human IL-12, human IL-12 p40, murine IL-12, and prestained molecular weight markers. FIG. **2A** shows bands stained from total protein. The primary bands in each lane are human IL-12 (75 kd), p40 human IL-12 (40 kd), and murine IL-12 (75 kd). FIG. **2B** shows a western blot prepared from a gel identical to that shown in FIG. **2A**. Blot was reacted with C340 followed by HRP labeled goat anti-human IgG and specifically detected human IL-12 (monomer and multimers) and human IL-12 p40 only. A control blot (not shown) reacted with HRP labeled goat anti-human IgG did not display any bands.

FIG. **3**: Reverse transcription-PCR analysis of IFNγ gene expression in human PBL's treated with IL-2, IL-12, IL-2+ IL-12 with and without anti-IL-12 antibody C340, 8.6.2, isotype control antibody. Total RNA was reverse transcribed, amplified by PCR using gene-specific primers. The level of β-actin mRNA in each sample was also determined which served as a control for mRNA integrity and content.

FIG. **4** is a histogram showing that human anti-IL-12 mAb (C340) inhibits production of interferon-γ (IFNγ) by monocyte depleted CD3+ peripheral blood mononuclear cells (PBMC) stimulated with IL-2 plus IL-12. PBMC were cultured for five hours in control media (no added cytokines), media supplemented with IL-12 (0.1 ng/ml) plus IL-2 (50 IU/ml) (IL-12/IL-2), control media containing mAb C340 (10 μg/ml) and IL-12/IL-2 media containing mAb C340 (10 μg/ml). Intracellular IFNγ was measured by two color immunostaining with CD3-PE and IFNγ-FITC. Data are shown for one donor.

FIG. **5** is a graph showing dose-dependent inhibition of IFNγ secretion by IL-2 plus IL-12 stimulated peripheral blood lymphocytes with two different lots of a human anti-IL-12 mAb (C340). Human PBL (8x106/ml) were cultured for 24 hours with 10 U/ml IL-2, IL-2 plus 400 pg/ml IL-12, or IL-2 plus IL-12 and mAb C340 as indicated. The culture supernatents were removed and assayed for IFNγ by EIA.

FIG. **6** is a histogram showing dose-dependent inhibition of IL-12 plus IL-2 induced LAK cell cytotoxicity by a

human anti-IL-12 mAb (C340). LAK effector cells (human PBL, 8x106/ml) were cultured for 24 hours with IL-12 (400 pg/ml) plus IL-2 (10 U/ml) and mAb C340 (5000 ng/ml or 50 ng/ml as indicated). The LAK effector cells were washed and cultured with 51Cr labeled Raji target cells for four hours at an effector to target (E:T) ration of 80:1, and the quantity of 51Cr released into the media upon Raji cell lysis was measured. Results are expressed as the mean of three normal donors standard error. IL-12 positive control (IL-12) is effector cells incubated with IL-12 and without antibody. Background (BKGD) is effector cells incubated without IL-12 or antibody.

FIGS. 7A and 7B are histograms showing that IL-12 plus IL-2-induced expression of CD95 on CD3+ peripheral blood mononuclear cells is inhibited by human anti-IL-12 mAb (C340). PBMC were cultured for 72 hours in media containing 0.1 ng/ml IL-12 and a suboptimal dose of IL-2 (50 IU/ml) in the presence or absence of mAb C340 (10 μg/ml). CD95 expression was measured flow cytometry of cells stained with anti-CD95-FITC. Gating was performed using two-color analysis (CD3 or CD56-PE vs. CD95-FITC) and forward vs. orthogonal light scatter.

FIG. 8 is a graph showing that recombinant human anti-human IL-12 antibodies (rC340) bind to immobilized IL-12 in a manner that is indistinguishable from purified mAb C340. The concentration of rC340 in the supernatants of three rC340-producing recombinant cell lines was determined, and the supernatants were evaluated for IL-12 binding in an ELISA. Plates were coated with 2 μg/ml human IL-12 and incubated with purified mAb C340 from the original hybridoma (standard) or the supernatants of recombinant cell lines. IL-12-bound antibody was detected using alkaline phosphatase-conjugated goat anti-human IgG (heavy chain+light chain).

FIGS. 9A–9C are graphs showing growth kinetics and the quantity of antibody secreted by three independently-derived rC340-producing recombinant cell subclones (FIG. 9A, subclone C379B; FIG. 9B, subclone C381A; FIG. 9C, subclone C389A). Recombinant cells were seeded into T75 flasks at a starting density of 2×10^5 cells/ml in standard media. At various times, cells were resuspended and the number of live cells and the quantity (μg/ml) of rC340 in the media were determined.

DESCRIPTION OF THE INVENTION

The present invention provides isolated, recombinant and/or synthetic anti-IL-12 human, primate, rodent, mammalian, chimeric, humanized or CDR-grafted, antibodies and IL-12 anti-idiotype antibodies thereto, as well as compositions and encoding nucleic acid molecules comprising at least one polynucleotide encoding at least one anti-IL-12 antibody or anti-idiotype antibody. The present invention further includes, but is not limited to, methods of making and using such nucleic acids and antibodies and anti-idiotype antibodies, including diagnostic and therapeutic compositions, methods and devices.

As used herein, an "anti-Interleukin-12 antibody," "anti-IL-12 antibody," "anti-IL-12 antibody portion," or "anti-IL-12 antibody fragment" and/or "anti-IL-12 antibody variant" and the like include any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to at least one complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, or at least one portion of an IL-12 receptor or binding protein, which can be incorporated into an antibody of the present invention. Such antibody optionally further affects a specific ligand, such as but not limited to where such antibody modulates, decreases, increases, antagonizes, agonizes, mitigates, alleviates, blocks, inhibits, abrogates and/or interferes with at least one IL-12 activity or binding, or with IL-12 receptor activity or binding, in vitro, in situ and/or in vivo. As a non-limiting example, a suitable anti-IL-12 antibody, specified portion or variant of the present invention can bind at least one IL-12, or specified portions, variants or domains thereof. A suitable anti-IIL-12 antibody, specified portion, or variant can also optionally affect at least one of IL-12 activity or function, such as but not limited to, RNA, DNA or protein synthesis, IL-12 release, IL-12 receptor signaling, membrane IL-12 cleavage, IL-12 activity, IL-12 production and/or synthesis. The term "antibody" is further intended to encompass antibodies, digestion fragments, specified portions and variants thereof, including antibody mimetics or comprising portions of antibodies that mimic the structure and/or function of an antibody or specified fragment or portion thereof, including single chain antibodies and fragments thereof. Functional fragments include antigen-binding fragments that bind to a mammalian IL-12. For example, antibody fragments capable of binding to IL-12 or portions thereof, including, but not limited to Fab (e.g., by papain digestion), Fab' (e.g., by pepsin digestion and partial reduction) and $F(ab')_2$ (e.g., by pepsin digestion), facb (e.g., by plasmin digestion), pFc' (e.g., by pepsin or plasmin digestion), Fd (e.g., by pepsin digestion, partial reduction and reaggregation), Fv or scFv (e.g., by molecular biology techniques) fragments, are encompassed by the invention (see, e.g., Colligan, Immunology, supra).

Such fragments can be produced by enzymatic cleavage, synthetic or recombinant techniques, as known in the art and/or as described herein. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a combination gene encoding a $F(ab')_2$ heavy chain portion can be designed to include DNA sequences encoding the CH_1 domain and/or hinge region of the heavy chain. The various portions of antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques.

As used herein, the term "human antibody" refers to an antibody in which substantially every part of the protein (e.g., CDR, framework, C_L, C_H domains (e.g., C_H1, C_H2, C_H3), hinge, (V_L, V_H)) is substantially non-immunogenic in humans, with only minor sequence changes or variations. Similarly, antibodies designated primate (monkey, babboon, chimpanzee, etc.), rodent (mouse, rat, rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-genus, genus, sub-family, family specific antibodies. Further, chimeric antibodies include any combination of the above. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a human antibody is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, an Fv can comprise a linker peptide, such as two to about eight glycine

or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

Bispecific, heterospecific, heteroconjugate or similar antibodies can also be used that are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for at least one IL-12 protein, the other one is for any other antigen. Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature 305:537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed, e.g., in WO 93/08829, U.S. Pat. Nos. 6,210,668, 6,193,967, 6,132,992, 6,106,833, 6,060,285, 6,037,453, 6,010,902, 5,989,530, 5,959,084, 5,959,083, 5,932,448, 5,833,985, 5,821,333, 5,807,706, 5,643,759, 5,601,819, 5,582,996, 5,496,549, 4,676,980, WO 91/00360, WO 92/00373, EP 03089, Traunecker et al., EMBO J. 10:3655 (1991), Suresh et al., Methods in Enzymology 121:210 (1986), each entirely incorporated herein by reference.

Anti-IL-12 antibodies (also termed IL-12 antibodies) useful in the methods and compositions of the present invention can optionally be characterized by high affinity binding to IL-12 and optionally and preferably having low toxicity. In particular, an antibody, specified fragment or variant of the invention, where the individual components, such as the variable region, constant region and framework, individually and/or collectively, optionally and preferably possess low immunogenicity, is useful in the present invention. The antibodies that can be used in the invention are optionally characterized by their ability to treat patients for extended periods with measurable alleviation of symptoms and low and/or acceptable toxicity. Low or acceptable immunogenicity and/or high affinity, as well as other suitable properties, can contribute to the therapeutic results achieved. "Low immunogenicity" is defined herein as raising significant HAHA, HACA or HAMA responses in less than about 75%, or preferably less than about 50% of the patients treated and/or raising low titres in the patient treated (less than about 300, preferably less than about 100 measured with a double antigen enzyme immunoassay) (see, e.g., Elliott et al., *Lancet* 344:1125–1127 (1994), entirely incorporated herein by reference).

Utility

The isolated nucleic acids of the present invention can be used for production of at least one anti-IL-12 antibody or specified variant thereof, which can be used to measure or effect in an cell, tissue, organ or animal (including mammals and humans), to diagnose, monitor, modulate, treat, alleviate, help prevent the incidence of, or reduce the symptoms of, at least one IL-12 condition, selected from, but not limited to, at least one of an immune disorder or disease, a cardiovascular disorder or disease, an infectious, malignant, and/or neurologic disorder or disease, or other known or specified IL-12 related condition.

Such a method can comprise administering an effective amount of a composition or a pharmaceutical composition comprising at least one anti-IL-12 antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment, alleviation, prevention, or reduction in symptoms, effects or mechanisms. The effective amount can comprise an amount of about 0.001 to 500 mg/kg per single (e.g., bolus), multiple or continuous administration, or to achieve a serum concentration of 0.01–5000 μg/ml serum concentration per single, multiple or continuous adminstration, or any effective range or value therein, as done and determined using known methods, as described herein or known in the relevant arts.

Citations

All publications or patents cited herein are entirely incorporated herein by reference as they show the state of the art at the time of the present invention and/or to provide description and enablement of the present invention. Publications refer to any scientific or patent publications, or any other information available in any media format, including all recorded, electronic or printed formats. The following references are entirely incorporated herein by reference: Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, N.Y. (1987–2001); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, N.Y. (1989); Harlow and Lane, antibodies, a Laboratory Manual, Cold Spring Harbor, N.Y. (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994–2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, New York, N.Y., (1997–2001).

Antibodies of the Present Invention

At least one anti-IL-12 antibody of the present invention can be optionally produced by a cell line, a mixed cell line, an immortalized cell or clonal population of immortalized cells, as well known in the art. See, e.g., Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York, N.Y. (1987–2001); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, N.Y. (1989); Harlow and Lane, antibodies, a Laboratory Manual, Cold Spring Harbor, N.Y. (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994–2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, New York, N.Y., (1997–2001), each entirely incorporated herein by reference.

Human antibodies that are specific for human IL-12 proteins or fragments thereof can be raised against an appropriate immunogenic antigen, such as isolated and/or IL-12 protein or a portion thereof (including synthetic molecules, such as synthetic peptides). Other specific or general mammalian antibodies can be similarly raised. Preparation of immunogenic antigens, and monoclonal antibody production can be performed using any suitable technique.

In one approach, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-AG14, NSO, NS1, NS2, AE-1, L.5, >243, P3X63Ag8.653, Sp2 SA3, Sp2 MAI, Sp2 SS1, Sp2 SA5, U937, MLA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMAIWA, NEURO 2A, or the like, or heteromylomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art. See, e.g., www.atcc.org, www.lifetech.com., and the like, with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood, lymph, tonsil, or other immune or B cell containing cells, or any other cells expressing heavy or light

chain constant or variable or framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA, single, double or triple stranded, hybridized, and the like or any combination thereof. See, e.g., Ausubel, supra, and Colligan, Immunology, supra, chapter 2, entirely incorporated herein by reference.

Antibody producing cells can also be obtained from the peripheral blood or, preferably the spleen or lymph nodes, of humans or other suitable animals that have been immunized with the antigen of interest. Any other suitable host cell can also be used for expressing heterologous or endogenous nucleic acid encoding an antibody, specified fragment or variant thereof, of the present invention. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods. Cells which produce antibodies with the desired specificity can be selected by a suitable assay (e.g., ELISA).

Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or protein library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, or the like, display library; e.g., as available from Cambridge antibody Technologies, Cambridgeshire, UK; MorphoSys, Martinsreid/Planegg, DE; Biovation, Aberdeen, Scotland, UK; BioInvent, Lund, Sweden; Dyax Corp., Enzon, Affymax/Biosite; Xoma, Berkeley, Calif.; Ixsys. See, e.g., EP 368,684, PCT/GB91/01134; PCT/GB92/01755; PCT/GB92/002240; PCT/GB92/00883; PCT/GB93/00605; U.S. Ser. No. 08/350,260 (May 12, 1994); PCT/GB94/01422; PCT/GB94/02662; PCT/GB97/01835; (CAT/MRC); WO90/14443; WO90/14424; WO90/14430; PCT/US94/1234; WO92/18619; WO96/07754; (Scripps); EP 614 989 (MorphoSys); WO95/16027 (BioInvent); WO88/06630; WO90/3809 (Dyax); U.S. Pat. No. 4,704,692 (Enzon); PCT/US91/02989 (Affymax); WO89/06283; EP 371 998; EP 550 400; (Xoma); EP 229 046; PCT/US91/07149 (Ixsys); or stochastically generated peptides or proteins—U.S. Pat. Nos. 5,723,323, 5,763,192, 5,814,476, 5,817,483, 5,824,514, 5,976,862, WO 86/05803, EP 590 689 (Ixsys, now Applied Molecular Evolution (AME), each entirely incorporated herein by reference) or that rely upon immunization of transgenic animals (e.g., SCID mice, Nguyen et al., Microbiol. Immunol. 41:901–907 (1997); Sandhu et al., Crit. Rev. Biotechnol. 16:95–118 (1996); Eren et al., Immunol. 93:154–161 (1998), each entirely incorporated by reference as well as related patents and application) that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein. Such techniques, include, but are not limited to, ribosome display (Hanes et al., Proc. Natl. Acad. Sci. USA, 94:4937–4942 (May 1997); Hanes et al., Proc. Natl. Acad. Sci. USA, 95:14130–14135 (November 1998)); single cell antibody producing technologies (e.g., selected lymphocyte antibody method ("SLAM") (U.S. Pat. No. 5,627,052, Wen et al., J. Immunol. 17:887–892 (1987); Babcook et al., Proc. Natl. Acad. Sci. USA 93:7843–7848 (1996)); gel microdroplet and flow cytometry (Powell et al., Biotechnol. 8:333–337 (1990); One Cell Systems, Cambridge, Mass.; Gray et al., J. Imm. Meth. 182:155–163 (1995); Kenny et al., Bio/Technol. 13:787–790 (1995)); B-cell selection (Steenbakkers et al., Molec. Biol. Reports 19:125–134 (1994); Jonak et al., Progress Biotech, Vol. 5, In Vitro Immunization in Hybridoma Technology, Borrebaeck, ed., Elsevier Science Publishers B. V., Amsterdam, Netherlands (1988)).

Methods for engineering or humanizing non-human or human antibodies can also be used and are well known in the art. Generally, a humanized or engineered antibody has one or more amino acid residues from a source which is non-human, e.g., but not limited to mouse, rat, rabbit, non-human primate or other mammal. These human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable, constant or other domain of a known human sequence. Known human Ig sequences are disclosed, e.g., www.ncbi.nlm.nih.gov/entrez/query.fcgi; www.atcc.org/phage/hdb.html; www.sciquest.com/; www.abcam.com/; www.antibodyresource.com/onlinecomp.html; www.public.iastate.edu/~pedro/research_tools.html; www.mgen.uni-heidelberg.de/SD/IT/IT.html; www.whfreeman.com/immunology/CH05/kuby05.htm; www.library.thinkquest.org/12429/Immune/Antibody.html; www.hhmi.org/grants/lectures/1996/vlab/; www.path.cam.ac.uk/~mrc7/mikeimages.html; www.antibodyresource.com/; mcb.harvard.edu/BioLinks/Immunology.html.www.immunologylink.com/; pathbox.wustl.edu/~hcenter/index.html; www.biotech.ufl.edu/-hcl/; www.pebio.com/pa/340913/340913.html; www.nal.usda.gov/awic/pubs/antibody/; www.m.ehime-u.ac.jp/~yasuhito/Elisa.html; www.biodesign.com/table.asp; www.icnet.uk/axp/facs/davies/links.html; www.biotech.ufl.edu/~fccl/protocol.html; www.isac-net.org/sites_geo.html; aximtl.imt.uni-marburg.de/~rek/AEPStart.html; baserv.uci.kun.nl/~jraats/links1.html; www.recab.uni-hd.de/immuno.bme.nwu.edu/; www.mrc-cpe.cam.ac.uk/imt-doc/public/INTRO.html; www.ibt.unam.mx/vir/V_mice.html; imgt.cnusc.fr:8104/; www.biochem.ucl.ac.uk/~martin/abs/index.html; antibody.bath.ac.uk/; abgen.cvm.tamu.edu/lab/wwwabgen.html; www.unizh.ch/~honegger/AHOseminar/Slide01.html; www.cryst.bbk.ac.uk/~ubcg07s/; www.nimr.mrc.ac.uk/CC/ccaewg/ccaewg.htm; www.path.cam.ac.uk/~mrc7/humanisation/TAHHP.html; www.ibt.unam.mx/vir/structure/stat_aim.html; www.biosci.missouri.edu/smithgp/index.html; www.cryst.bioc.cam.ac.uk/~fmolina/Web-pages/Pept/spottech.html; www.jerini.de/fr_products.htm; www.patents.ibm.com/ibm.html.Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983), each entirely incorporated herein by reference. Such imported sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art. Generally part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids. Antibodies can also optionally be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, humanized antibodies can be optionally prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin

sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Humanization or engineering of antibodies of the present invention can be performed using any known method, such as but not limited to those described in, Winter (Jones et al., Nature 321:522 (1986); Riechmann et al., Nature 332:323 (1988); Verhoeyen et al., Science 239: 1534 (1988)), Sims et al., J. Immunol. 151: 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987), Carter et al., Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993), U.S. Pat. Nos: 5,723,323, 5,976,862, 5,824,514, 5,817,483, 5,814,476, 5,763,192, 5,723,323, 5,766,886, 5,714,352, 6,204,023, 6,180,370, 5,693,762, 5,530,101, 5,585,089, 5,225,539; 4,816,567, PCT/: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB89/01334, GB91/01134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, each entirely incorporated herein by reference, included references cited therein.

The anti-IL-12 antibody can also be optionally generated by immunization of a transgenic animal (e.g., mouse, rat, hamster, non-human primate, and the like) capable of producing a repertoire of human antibodies, as described herein and/or as known in the art. Cells that produce a human anti-IL-12 antibody can be isolated from such animals and immortalized using suitable methods, such as the methods described herein.

Transgenic mice that can produce a repertoire of human antibodies that bind to human antigens can be produced by known methods (e.g., but not limited to, U.S. Pat. Nos. 5,770,428, 5,569,825, 5,545,806, 5,625,126, 5,625,825, 5,633,425, 5,661,016 and 5,789,650 issued to Lonberg et al.; Jakobovits et al. WO 98/50433, Jakobovits et al. WO 98/24893, Lonberg et al. WO 98/24884, Lonberg et al. WO 97/13852, Lonberg et al. WO 94/25585, Kucherlapate et al. WO 96/34096, Kucherlapate et al. EP 0463 151 B1, Kucherlapate et al. EP 0710 719 A1, Surani et al. U.S. Pat. No. 5,545,807, Bruggemann et al. WO 90/04036, Bruggemann et al. EP 0438 474 B1, Lonberg et al. EP 0814 259 A2, Lonberg et al. GB 2 272 440 A, Lonberg et al. *Nature* 368:856–859 (1994), Taylor et al., *Int. Immunol.* 6(4) 579–591 (1994), Green et al, *Nature Genetics* 7:13–21 (1994), Mendez et al., *Nature Genetics* 15:146–156 (1997), Taylor et al., *Nucleic Acids Research* 20(23):6287–6295 (1992), Tuaillon et al., *Proc Natl Acad Sci USA* 90(8) 3720–3724 (1993), Lonberg et al., *Int Rev Immunol* 13(1): 65–93 (1995) and Fishwald et al., *Nat Biotechnol* 14(7): 845–851 (1996), which are each entirely incorporated herein by reference). Generally, these mice comprise at least one transgene comprising DNA from at least one human immunoglobulin locus that is functionally rearranged, or which can undergo functional rearrangement. The endogenous immunoglobulin loci in such mice can be disrupted or deleted to eliminate the capacity of the animal to produce antibodies encoded by endogenous genes.

Screening antibodies for specific binding to similar proteins or fragments can be conveniently achieved using peptide display libraries. This method involves the screening of large collections of peptides for individual members having the desired function or structure. Antibody screening of peptide display libraries is well known in the art. The displayed peptide sequences can be from 3 to 5000 or more amino acids in length, frequently from 5–100 amino acids long, and often from about 8 to 25 amino acids long. In addition to direct chemical synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. Such methods are described in PCT Patent Publication Nos. 91/17271, 91/18980, 91/19818, and 93/08278. Other systems for generating libraries of peptides have aspects of both in vitro chemical synthesis and recombinant methods. See, PCT Patent Publication Nos. 92/05258, 92/14843, and 96/19256. See also, U.S. Pat. Nos. 5,658,754; and 5,643,768. Peptide display libraries, vector, and screening kits are commercially available from such suppliers as Invitrogen (Carlsbad, Calif.), and Cambridge Antibody Technologies (Cambridgeshire, UK). See, e.g., U.S. Pat. Nos. 4,704,692, 4,939,666, 4,946,778, 5,260,203, 5,455,030, 5,518,889, 5,534,621, 5,656,730, 5,763,733, 5,767,260, 5,856,456, assigned to Enzon; U.S. Pat. Nos. 5,223,409, 5,403,484, 5,571,698, 5,837,500, assigned to Dyax, U.S. Pat. Nos. 5,427,908, 5,580,717, assigned to Affymax; U.S. Pat. No. 5,885,793, assigned to Cambridge antibody Technologies; U.S. Pat. No. 5,750,373, assigned to Genentech, U.S. Pat. Nos. 5,618,920, 5,595,898, 5,576,195, 5,698,435, 5,693,493, 5,698,417, assigned to Xoma, Colligan, supra; Ausubel, supra; or Sambrook, supra, each of the above patents and publications entirely incorporated herein by reference.

Antibodies of the present invention can also be prepared using at least one anti-IL-12 antibody encoding nucleic acid to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such antibodies in their milk. Such animals can be provided using known methods. See, e.g., but not limited to, U.S. Pat. Nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; 5,304,489, and the like, each of which is entirely incorporated herein by reference.

Antibodies of the present invention can additionally be prepared using at least one anti-IL-12 antibody encoding nucleic acid to provide transgenic plants and cultured plant cells (e.g., but not limited to tobacco and maize) that produce such antibodies, specified portions or variants in the plant parts or in cells cultured therefrom. As a non-limiting example, transgenic tobacco leaves expressing recombinant proteins have been successfully used to provide large amounts of recombinant proteins, e.g., using an inducible promoter. See, e.g., Cramer et al., Curr. Top. Microbol. Immunol. 240:95–118 (1999) and references cited therein. Also, transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. See, e.g., Hood et al., Adv. Exp. Med. Biol. 464:127–147 (1999) and references cited therein. Antibodies have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as single chain antibodies (scFv's), including tobacco seeds and potato tubers. See, e.g., Conrad et al., Plant Mol. Biol. 38:101–109 (1998) and reference cited therein. Thus, antibodies of the present invention can also be produced using transgenic plants, according to know methods. See also, e.g., Fischer et al., Biotechnol. Appl. Biochem. 30:99–108 (October, 1999), Ma et al., Trends Biotechnol. 13:522–7 (1995); Ma et al., Plant Physiol. 109:341–6 (1995); Whitelam et al., Biochem. Soc. Trans.

22:940–944 (1994); and references cited therein. Each of the above references is entirely incorporated herein by reference.

The antibodies of the invention can bind human IL-12 with a wide range of affinities (K_D). In a preferred embodiment, at least one human mAb of the present invention can optionally bind human IL-12 with high affinity. For example, a human mAb can bind human IL-12 with a K_D equal to or less than about 10^{-7} M, such as but not limited to, 0.1–9.9 (or any range or value therein) X 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, 10^{-12}, 10^{-13} or any range or value therein.

The affinity or avidity of an antibody for an antigen can be determined experimentally using any suitable method. (See, for example, Berzofsky, et al., "Antibody-Antigen Interactions," In *Fundamental Immunology*, Paul, W. E., Ed., Raven Press: New York, N.Y. (1984); Kuby, Janis *Immunology*, W. H. Freeman and Company: New York, N.Y. (1992); and methods described herein). The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity and other antigen-binding parameters (e.g., K_D, K_a, K_d) are preferably made with standardized solutions of antibody and antigen, and a standardized buffer, such as the buffer described herein.

Nucleic Acid Molecules

Using the information provided herein, such as the nucleotide sequences encoding at least 70–100% of the contiguous amino acids of at least one of SEQ ID NOS:1, 2, 3, 4, 5, 6, 7, 8, specified fragments, variants or consensus sequences thereof, or a deposited vector comprising at least one of these sequences, a nucleic acid molecule of the present invention encoding at least one anti-IL-12 antibody can be obtained using methods described herein or as known in the art.

Nucleic acid molecules of the present invention can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or produced synthetically, or any combinations thereof. The DNA can be triple-stranded, double-stranded or single-stranded, or any combination thereof. Any portion of at least one strand of the DNA or RNA can be the coding strand, also known as the sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.

Isolated nucleic acid molecules of the present invention can include nucleic acid molecules comprising an open reading frame (ORF), optionally with one or more introns, e.g., but not limited to, at least one specified portion of at least one CDR, as GDR1, CDR2 and/or CDR3 of at least one heavy chain (e.g., SEQ ID NOS: 1–3) or light chain (e.g., SEQ ID NOS: 4–6); nucleic acid molecules comprising the coding sequence for an anti-IL-12 antibody or variable region (e.g., SEQ ID NOS:7,8); and nucleic acid molecules which comprise a nucleotide sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode at least one anti-IL-12 antibody as described herein and/or as known in the art. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate nucleic acid variants that code for specific anti-IL-12 antibodies of the present invention. See, e.g., Ausubel, et al., supra, and such nucleic acid variants are included in the present invention. Non-limiting examples of isolated nucleic acid molecules of the present invention include SEQ ID NOS:10–15, corresponding to non-limiting examples of a nucleic acid encoding, respectively, HG CDR1, HG CDR2, HG CDR3, LC CDR1, LC CDR2, LC CDR3, HG variable region and LC variable region.

As indicated herein, nucleic acid molecules of the present invention which comprise a nucleic acid encoding an anti-IL-12 antibody can include, but are not limited to, those encoding the amino acid sequence of an antibody fragment, by itself; the coding sequence for the entire antibody or a portion thereof; the coding sequence for an antibody, fragment or portion, as well as additional sequences, such as the coding sequence of at least one signal leader or fusion peptide, with or without the aforementioned additional coding sequences, such as at least one intron, together with additional, non-coding sequences, including but not limited to, non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals (for example—ribosome binding and stability of mRNA); an additional coding sequence that codes for additional amino acids, such as those that provide additional functionalities. Thus, the sequence encoding an antibody can be fused to a marker sequence, such as a sequence encoding a peptide that facilitates purification of the fused antibody comprising an antibody fragment or portion.

Polynucleotides Which Selectively Hybridize to a Polynucleotide as Described Herein

The present invention provides isolated nucleic acids that hybridize under selective hybridization conditions to a polynucleotide disclosed herein. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising such polynucleotides. For example, polynucleotides of the present invention can be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In some embodiments, the polynucleotides are genomic or cDNA sequences isolated, or otherwise complementary to, a cDNA from a human or mammalian nucleic acid library.

Preferably, the cDNA library comprises at least 80% full-length sequences, preferably at least 85% or 90% full-length sequences, and more preferably at least 95% full-length sequences. The cDNA libraries can be normalized to increase the representation of rare sequences. Low or moderate stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 70% sequence identity and can be employed to identify orthologous or paralogous sequences.

Optionally, polynucleotides of this invention will encode at least a portion of an antibody encoded by the polynucleotides described herein. The polynucleotides of this invention embrace nucleic acid sequences that can be employed for selective hybridization to a polynucleotide encoding an antibody of the present invention. See, e.g., Ausubel, supra; Colligan, supra, each entirely incorporated herein by reference.

Construction of Nucleic Acids

The isolated nucleic acids of the present invention can be made using (a) recombinant methods, (b) synthetic techniques, (c) purification techniques, or combinations thereof, as well-known in the art.

The nucleic acids can conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites can be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable

sequences can be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the present invention. The nucleic acid of the present invention—excluding the coding sequence—is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention.

Additional sequences can be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. (See, e.g., Ausubel, supra; or Sambrook, supra)

Recombinant Methods for Constructing Nucleic Acids

The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA, or any combination thereof, can be obtained from biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes that selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. The isolation of RNA, and construction of cDNA and genomic libraries, is well known to those of ordinary skill in the art. (See, e.g., Ausubel, supra; or Sambrook, supra)

Nucleic Acid Screening and Isolation Methods

A cDNA or genomic library can be screened using a probe based upon the sequence of a polynucleotide of the present invention, such as those disclosed herein. Probes can be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different organisms. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity between the probe and the target for duplex formation to occur. The degree of stringency can be controlled by one or more of temperature, ionic strength, pH and the presence of a partially denaturing solvent such as formamide. For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant solution through, for example, manipulation of the concentration of formamide within the range of 0% to 50%. The degree of complementarity (sequence identity) required for detectable binding will vary in accordance with the stringency of the hybridization medium and/or wash medium. The degree of complementarity will optimally be 100%, or 70–100%, or any range or value therein. However, it should be understood that minor sequence variations in the probes and primers can be compensated for by reducing the stringency of the hybridization and/or wash medium.

Methods of amplification of RNA or DNA are well known in the art and can be used according to the present invention without undue experimentation, based on the teaching and guidance presented herein.

Known methods of DNA or RNA amplification include, but are not limited to, polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. Pat. Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis, et al.; U.S. Pat. Nos. 4,795,699 and 4,921,794 to Tabor, et al; U.S. Pat. No. 5,142,033 to Innis; U.S. Pat. No. 5,122,464 to Wilson, et al.; U.S. Pat. No. 5,091,310 to Innis; U.S. Pat. No. 5,066,584 to Gyllensten, et al; U.S. Pat. No. 4,889,818 to Gelfand, et al; U.S. Pat. No. 4,994,370 to Silver, et al; U.S. Pat. No. 4,766,067 to Biswas; U.S. Pat. No. 4,656,134 to Ringold) and RNA mediated amplification that uses antisense RNA to the target sequence as a template for double-stranded DNA synthesis (U.S. Pat. No. 5,130,238 to Malek, et al, with the tradename NASBA), the entire contents of which references are incorporated herein by reference. (See, e.g., Ausubel, supra; or Sambrook, supra.)

For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the present invention and related genes directly from genomic DNA or cDNA libraries. PCR and other in vitro amplification methods can also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through in vitro amplification methods are found in Berger, supra, Sambrook, supra, and Ausubel, supra, as well as Mullis, et al., U.S. Pat. No. 4,683,202 (1987); and Innis, et al., PCR Protocols A Guide to Methods and Applications, Eds., Academic Press Inc., San Diego, Calif. (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). Additionally, e.g., the T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

Synthetic Methods for Constructing Nucleic Acids

The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis by known methods (see, e.g., Ausubel, et al., supra). Chemical synthesis generally produces a single-stranded oligonucleotide, which can be converted into double-stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill in the art will recognize that while chemical synthesis of DNA can be limited to sequences of about 100 or more bases, longer sequences can be obtained by the ligation of shorter sequences.

Recombinant Expression Cassettes

The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence of the present invention, for example a cDNA or a genomic sequence encoding an antibody of the present invention, can be used to construct a recombinant expression cassette that can be introduced into at least one desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention operably linked to transcriptional initiation regulatory sequences that will direct the transcription of the polynucleotide in the intended host cell. Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention.

In some embodiments, isolated nucleic acids that serve as promoter, enhancer, or other elements can be introduced in the appropriate position (upstream, downstream or in intron) of a non-heterologous form of a polynucleotide of the present invention so as to up or down regulate expression of a polynucleotide of the present invention. For example, endogenous promoters can be altered in vivo or in vitro by mutation, deletion and/or substitution.

Vectors and Host Cells

The present invention also relates to vectors that include isolated nucleic acid molecules of the present invention, host cells that are genetically engineered with the recombinant vectors, and the production of at least one anti-IL-12 anti-

body by recombinant techniques, as is well known in the art. See, e.g., Sambrook, et al., supra; Ausubel, et al., supra, each entirely incorporated herein by reference.

The polynucleotides can optionally be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it can be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (e.g., UAA, UGA or UAG) appropriately positioned at the end of the mRNA to be translated, with UAA and UAG preferred for mammalian or eukaryotic cell expression.

Expression vectors will preferably but optionally include at least one selectable marker. Such markers include, e.g., but not limited to, methotrexate (MTX), dihydrofolate reductase (DHFR, U.S. Pat. Nos. 4,399,216; 4,634,665; 4,656,134; 4,956,288; 5,149,636; 5,179,017), ampicillin, neomycin (G418), mycophenolic acid, or glutamine synthetase (GS, U.S. Pat. Nos. 5,122,464; 5,770,359; 5,827,739) resistance for eukaryotic cell culture, and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria or prokaryotics (the above patents are entirely incorporated hereby by reference). Appropriate culture mediums and conditions for the above-described host cells are known in the art. Suitable vectors will be readily apparent to the skilled artisan. Introduction of a vector construct into a host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other known methods. Such methods are described in the art, such as Sambrook, supra, Chapters 1–4 and 16–18; Ausubel, supra, Chapters 1, 9, 13, 15, 16.

At least one antibody of the present invention can be expressed in a modified form, such as a fusion protein, and can include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of an antibody to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to an antibody of the present invention to facilitate purification. Such regions can be removed prior to final preparation of an antibody or at least one fragment thereof. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Chapters 17.29–17.42 and 18.1–18.74; Ausubel, supra, Chapters 16, 17 and 18.

Those of ordinary skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention.

Alternatively, nucleic acids of the present invention can be expressed in a host cell by turning on (by manipulation) in a host cell that contains endogenous DNA encoding an antibody of the present invention. Such methods are well known in the art, e.g., as described in U.S. Pat. Nos. 5,580,734, 5,641,670, 5,733,746, and 5,733,761, entirely incorporated herein by reference.

Illustrative of cell cultures useful for the production of the antibodies, specified portions or variants thereof, are mammalian cells. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions or bioreactors can also be used. A number of suitable host cell lines capable of expressing intact glycosylated proteins have been developed in the art, and include the COS-1 (e.g., ATCC CRL 1650), COS-7 (e.g., ATCC CRL-1651), HEK293, BHK21 (e.g., ATCC CRL-10), CHO (e.g., ATCC CRL 1610) and BSC-1 (e.g., ATCC CRL-26) cell lines, Cos-7 cells, CHO cells, hep G2 cells, P3X63Ag8.653, SP2/0-Ag14, 293 cells, HeLa cells and the like, which are readily available from, for example, American Type Culture Collection, Manassas, Va. (www.atcc.org). Preferred host cells include cells of lymphoid origin such as myeloma and lymphoma cells. Particularly preferred host cells are P3X63Ag8.653 cells (ATCC Accession Number CRL-1580) and SP2/0-Ag14 cells (ATCC Accession Number CRL-1851). In a particularly preferred embodiment, the recombinant cell is a P3X63Ab8.653 or a SP2/0-Ag14 cell.

Expression vectors for these cells can include one or more of the following expression control sequences, such as, but not limited to an origin of replication; a promoter (e.g., late or early SV40 promoters, the CMV promoter (U.S. Pat. Nos. 5,168,062; 5,385,839), an HSV tk promoter, a pgk (phosphoglycerate kinase) promoter, an EF-1 alpha promoter (U.S. Pat. No. 5,266,491), at least one human immunoglobulin promoter; an enhancer, and/or processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. See, e.g., Ausubel et al., supra; Sambrook, et al., supra. Other cells useful for production of nucleic acids or proteins of the present invention are known and/or available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (www.atcc.org) or other known or commercial sources.

When eukaryotic host cells are employed, polyadenlyation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenlyation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript can also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., J. Virol. 45:773–781 (1983)). Additionally, gene sequences to control replication in the host cell can be incorporated into the vector, as known in the art.

Purification of an Antibody

An anti-IL-12 antibody can be recovered and purified from recombinant cell cultures by well-known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be employed for purification. See, e.g., Colligan, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, New York, N.Y., (1997–2001), e.g., Chapters 1, 4, 6, 8, 9, 10, each entirely incorporated herein by reference.

Antibodies of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the antibody of the present invention can be glycosylated or can be non-glycosylated, with glycosylated preferred. Such

methods are described in many standard laboratory manuals, such as Sambrook, supra, Sections 17.37–17.42; Ausubel, supra, Chapters 10, 12, 13, 16, 18 and 20, Colligan, Protein Science, supra, Chapters 12–14, all entirely incorporated herein by reference.

Anti-IL-12 Antibodies

The isolated antibodies of the present invention comprise an antibody encoded by any one of the polynucleotides of the present invention as discussed more fully herein, or any isolated or prepared antibody. Preferably, the human antibody or antigen-binding fragment binds human IL-12 and, thereby partially or substantially neutralizes at least one biological activity of the protein. An antibody, or specified portion or variant thereof, that partially or preferably substantially neutralizes at least one biological activity of at least one IL-12 protein or fragment can bind the protein or fragment and thereby inhibit activities mediated through the binding of LL-12 to the LL-12 receptor or through other IL-12-dependent or mediated mechanisms. As used herein, the temi "neutralizing antibody" refers to an antibody that can inhibit an IL-12-dependent activity by about 20–120%, preferably by at least about 10, 20, 30, 40, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% or more depending on the assay. The capacity of an anti-IL-12 antibody to inhibit an IL-12-dependent activity is preferably assessed by at least one suitable IL-12 protein or receptor assay, as described herein and/or as known in the art. A human antibody of the invention can be of any class (IgG, IgA, IgM, IgE, IgD, etc.) or isotype and can comprise a kappa or lambda light chain. In one embodiment, the human antibody comprises an IgG heavy chain or defined fragment, for example, at least one of isotypes, IgG1, IgG2, IgG3 or IgG4. Antibodies of this type can be prepared by employing a transgenic mouse or other transgenic non-human mammal comprising at least one human light chain (e.g., IgG, IgA and IgM (e.g., γ1, γ2, γ3, γ4) transgenes as described herein and/or as known in the art. In another embodiment, the anti-human IL-12 human antibody comprises an IgG1 heavy chain and an IgG1 light chain.

At least one antibody of the invention binds at least one specified epitope specific to at least one IL-12 protein, subunit, fragment, portion or any combination thereof. The at least one epitope can comprise at least one antibody binding region that comprises at least one portion of said protein, which epitope is preferably comprised of at least one extracellular, soluble, hydrophillic, external or cytoplasmic portion of said protein. The at least one specified epitope can comprise any combination of at least one amino acid sequence of at least 1–3 amino acids to the entire specified portion of contiguous amino acids of the SEQ ID NO:9.

Generally, the human antibody or antigen-binding fragment of the present invention will comprise an antigen-binding region that comprises at least one human complementarity determining region (CDR1, CDR2 and CDR3) or variant of at least one heavy chain variable region and at least one human complementarity determining region (CDR1, CDR2 and CDR3) or variant of at least one light chain variable region. As a non-limiting example, the antibody or antigen-binding portion or variant can comprise at least one of the heavy chain CDR3 having the amino acid sequence of SEQ ID NO:3, and/or a light chain CDR3 having the amino acid sequence of SEQ ID NO:6. In a particular embodiment, the antibody or antigen-binding fragment can have an antigen-binding region that comprises at least a portion of at least one heavy chain CDR (i.e., CDR1, CDR2 and/or CDR3) having the amino acid sequence of the corresponding CDRs 1, 2 and/or 3 (e.g., SEQ ID NOS:1, 2, and/or 3). In another particular embodiment, the antibody or antigen-binding portion or variant can have an antigen-binding region that comprises at least a portion of at least one light chain CDR (i.e., CDR1, CDR2 and/or CDR3) having the amino acid sequence of the corresponding CDRs 1, 2 and/or 3 (e.g., SEQ ID NOS: 4, 5, and/or 6). In a preferred embodiment the three heavy chain CDRs and the three light chain CDRs of the anitbody or antigen-binding fragment have the amino acid sequence of the corresponding CDR of at least one of mAb 12B75, C340, or any others as described herein. Such antibodies can be prepared by chemically joining together the various portions (e.g., CDRs, framework) of the antibody using conventional techniques, by preparing and expressing a (i.e., one or more) nucleic acid molecule that encodes the antibody using conventional techniques of recombinant DNA technology or by using any other suitable method.

The anti-IL-12 antibody can comprise at least one of a heavy or light chain variable region having a defined amino acid sequence. For example, in a preferred embodiment, the anti-IL-12 antibody comprises at least one of at least one heavy chain variable region, optionally having the amino acid sequence of SEQ ID NO:7 and/or at least one light chain variable region, optionally having the amino acid sequence of SEQ ID NO:8. Antibodies that bind to human IL-12 and that comprise a defined heavy or light chain variable region can be prepared using suitable methods, such as phage display (Katsube, Y., et al., *Int J Mol. Med*, 1(5):863–868 (1998)) or methods that employ transgenic animals, as known in the art and/or as described herein. For example, a transgenic mouse, comprising a functionally rearranged human immunoglobulin heavy chain transgene and a transgene comprising DNA from a human immunoglobulin light chain locus that can undergo functional rearrangement, can be immunized with human IL-12 or a fragment thereof to elicit the production of antibodies. If desired, the antibody producing cells can be isolated and hybridomas or other immortalized antibody-producing cells can be prepared as described herein and/or as known in the art. Alternatively, the antibody, specified portion or variant can be expressed using the encoding nucleic acid or portion thereof in a suitable host cell.

The invention also relates to antibodies, antigen-binding fragments, immunoglobulin chains and CDRs comprising amino acids in a sequence that is substantially the same as an amino acid sequence described herein. Preferably, such antibodies or antigen-binding fragments and antibodies comprising such chains or CDRs can bind human IL-12 with high affinity (e.g., K_D less than or equal to about 10^{-9} M). Amino acid sequences that are substantially the same as the sequences described herein include sequences comprising conservative amino acid substitutions, as well as amino acid deletions and/or insertions. A conservative amino acid substitution refers to the replacement of a first amino acid by a second amino acid that has chemical and/or physical properties (e.g, charge, structure, polarity, hydrophobicity/hydrophilicity) that are similar to those of the first amino acid. Conservative substitutions include replacement of one amino acid by another within the following groups: lysine (K), arginine (R) and histidine (H); aspartate (D) and glutamate (E); asparagine (N), glutamine (Q), serine (S), threonine (T), tyrosine (Y), K, R, H, D and E; alanine (A), valine (V), leucine (L), isoleucine (I), proline (P), phenylalanine (F), tryptophan (W), methionine (M), cysteine (C) and glycine (G); F, W and Y; C, S and T.

Amino Acid Codes

The amino acids that make up anti-IL-12 antibodies of the present invention are often abbreviated. The amino acid

designations can be indicated by designating the amino acid by its single letter code, its three letter code, name, or three nucleotide codon(s) as is well understood in the art (see Alberts, B., et al., Molecular Biology of The Cell, Third Ed., Garland Publishing, Inc., New York, 1994):

SINGLE LETTER CODE	THREE LETTER CODE	NAME	THREE NUCLEOTIDE CODON(S)
A	Ala	Alanine	GCA, GCC, GCG, GCU
C	Cys	Cysteine	UGC, UGU
D	Asp	Aspartic acid	GAC, GAU
E	Glu	Glutamic acid	GAA, GAG
F	Phe	Phenylanine	UUC, UUU
G	Gly	Glycine	GGA, GGC, GGG, GGU
H	His	Histidine	CAC, CAU
I	Ile	Isoleucine	AUA, AUC, AUU
K	Lys	Lysine	AAA, AAG
L	Leu	Leucine	UUA, UUG, CUA, CUC, CUG, CUU
M	Met	Methionine	AUG
N	Asn	Asparagine	AAC, AAU
P	Pro	Proline	CCA, CCC, CCG, CCU
Q	Gln	Glutamine	CAA, CAG
R	Arg	Arginine	AGA, AGG, CGA, CGC, CGG, CGU
S	Ser	Serine	AGC, AGU, UCA, UCC, UCG, UCU
T	Thr	Threonine	ACA, ACC, ACG, ACU
V	Val	Valine	GUA, GUC, GUG, GUU
W	Trp	Tryptophan	UGG
Y	Tyr	Tyrosine	UAC, UAU

An anti-IL-12 antibody of the present invention can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as specified herein.

Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions, insertions or deletions for any given anti-IL-12 Ig-derived protein, fragment or variant will not be more than 40, 30, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, such as 1–30 or any range or value therein, as specified herein.

Amino acids in an anti-IL-12 antibody of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (e.g., Ausubel, supra, Chapters 8, 15; Cunningham and Wells, Science 244:1081–1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity, such as, but not limited to at least one IL-12 neutralizing activity. Sites that are critical for antibody binding can also be identified by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith, et al., J. Mol. Biol. 224:899–904 (1992) and de Vos, et al., Science 255:306–312 (1992)).

Anti-IL-12 antibodies of the present invention can include, but are not limited to, at least one portion, sequence or combination selected from 5 to all of the contiguous amino acids of at least one of SEQ ID NOS:1, 2, 3, 4, 5, 6.

IL-12 antibodies or specified portions or variants of the present invention can include, but are not limited to, at least one portion, sequence or combination selected from at least 3–5 contiguous amino acids of SEQ ID NO:1, 5–17 contiguous amino acids of SEQ ID NO:2, 5–10 contiguous amino acids of SEQ ID NO:3, 5–11 contiguous amino acids of SEQ ID NO:4, 5–7 contiguous amino acids of SEQ ID NO:5; 5–9 contiguous amino acids of SEQ ID NO:6; Leu21, Lys76, Met83, Ser85 of SEQ ID NO:7.

A(n) anti-IL-12 antibody can further optionally comprise a polypeptide of at least one of 70–100% of 5, 17, 10, 11, 7, 9, 119, or 108 contiguous amino acids of at least one of SEQ ID NOS:1, 2, 3, 4, 5, 6, 7 or 8.

In one embodiment, the amino acid sequence of an immunoglobulin chain, or portion thereof (e.g., variable region, CDR) has about 70–100% identity (e.g., 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) to the amino acid sequence of the corresponding chain of at least one of SEQ ID NOS:7, 8. For example, the amino acid sequence of a light chain variable region can be compared with the sequence of SEQ ID NO:8, or the amino acid sequence of a heavy chain CDR3 can be compared with SEQ ID NO:3. Preferably, 70–100% amino acid identity (i.e., 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or any range or value therein) is determined using a suitable computer algorithm, as known in the art.

Exemplary heavy chain and light chain variable regions sequences are provided in SEQ ID NOS: 7 and 8. The antibodies of the present invention, or specified variants thereof, can comprise any number of contiguous amino acid residues from an antibody of the present invention, wherein that number is selected from the group of integers consisting of from 10–100% of the number of contiguous residues in an anti-IL-12 antibody. Optionally, this subsequence of contiguous amino acids is at least about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250 or more amino acids in length, or any range or value therein. Further, the number of such subsequences can be any integer selected from the group consisting of from 1 to 20, such as at least 2, 3, 4, or 5.

As those of skill will appreciate, the present invention includes at least one biologically active antibody of the present invention. Biologically active antibodies have a specific activity at least 20%, 30%, or 40%, and preferably at least 50%, 60%, or 70%, and most preferably at least 80%, 90%, or 95%–1000% of that of the native (non-synthetic), endogenous or related and known antibody. Methods of assaying and quantifying measures of enzymatic activity and substrate specificity, are well known to those of skill in the art.

In another aspect, the invention relates to human antibodies and antigen-binding fragments, as described herein, which are modified by the covalent attachment of an organic moiety. Such modification can produce an antibody or antigen-binding fragment with improved pharmacokinetic properties (e.g., increased in vivo serum half-life). The organic moiety can be a linear or branched hydrophilic polymeric group, fatty acid group, or fatty acid ester group. In particular embodiments, the hydrophilic polymeric group can have a molecular weight of about 800 to about 120,000 Daltons and can be a polyalkane glycol (e.g., polyethylene glycol (PEG), polypropylene glycol (PPG)), carbohydrate polymer, amino acid polymer or polyvinyl pyrolidone, and the fatty acid or fatty acid ester group can comprise from about eight to about forty carbon atoms.

The modified antibodies and antigen-binding fragments of the invention can comprise one or more organic moieties that are covalently bonded, directly or indirectly, to the antibody. Each organic moiety that is bonded to an antibody or antigen-binding fragment of the invention can independently be a hydrophilic polymeric group, a fatty acid group or a fatty acid ester group. As used herein, the term "fatty acid" encompasses mono-carboxylic acids and di-carboxylic

acids. A "hydrophilic polymeric group," as the term is used herein, refers to an organic polymer that is more soluble in water than in octane. For example, polylysine is more soluble in water than in octane. Thus, an antibody modified by the covalent attachment of polylysine is encompassed by the invention. Hydrophilic polymers suitable for modifying antibodies of the invention can be linear or branched and include, for example, polyalkane glycols (e.g., PEG, monomethoxy-polyethylene glycol (mPEG), PPG and the like), carbohydrates (e.g., dextran, cellulose, oligosaccharides, polysaccharides and the like), polymers of hydrophilic amino acids (e.g., polylysine, polyarginine, polyaspartate and the like), polyalkane oxides (e.g., polyethylene oxide, polypropylene oxide and the like) and polyvinyl pyrolidone. Preferably, the hydrophilic polymer that modifies the antibody of the invention has a molecular weight of about 800 to about 150,000 Daltons as a separate molecular entity. For example PEG_{5000} and $PEG_{20,000}$ wherein the subscript is the average molecular weight of the polymer in Daltons, can be used. The hydrophilic polymeric group can be substituted with one to about six alkyl, fatty acid or fatty acid ester groups. Hydrophilic polymers that are substituted with a fatty acid or fatty acid ester group can be prepared by employing suitable methods. For example, a polymer comprising an amine group can be coupled to a carboxylate of the fatty acid or fatty acid ester, and an activated carboxylate (e.g., activated with N,N-carbonyl diimidazole) on a fatty acid or fatty acid ester can be coupled to a hydroxyl group on a polymer.

Fatty acids and fatty acid esters suitable for modifying antibodies of the invention can be saturated or can contain one or more units of unsaturation. Fatty acids that are suitable for modifying antibodies of the invention include, for example, n-dodecanoate (C_{12}, laurate), n-tetradecanoate (C_{14}, myristate), n-octadecanoate (C_{18}, stearate), n-eicosanoate (C_{20}, arachidate), n-docosanoate (C_{22}, behenate), n-triacontanoate (C_{30}), n-tetracontanoate (C_{40}), cis-Δ9-octadecanoate (C_{18}, oleate), all cis-Δ5,8,11,14-eicosatetraenoate (C_{20}, arachidonate), octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic acid, and the like. Suitable fatty acid esters include monoesters of dicarboxylic acids that comprise a linear or branched lower alkyl group. The lower alkyl group can comprise from one to about twelve, preferably one to about six, carbon atoms.

The modified human antibodies and antigen-binding fragments can be prepared using suitable methods, such as by reaction with one or more modifying agents. A "modifying agent" as the term is used herein, refers to a suitable organic group (e.g., hydrophilic polymer, a fatty acid, a fatty acid ester) that comprises an activating group. An "activating group" is a chemical moiety or functional group that can, under appropriate conditions, react with a second chemical group thereby forming a covalent bond between the modifying agent and the second chemical group. For example, amine-reactive activating groups include electrophilic groups such as tosylate, mesylate, halo (chloro, bromo, fluoro, iodo), N-hydroxysuccinimidyl esters (NHS), and the like. Activating groups that can react with thiols include, for example, maleimide, iodoacetyl, acrylolyl, pyridyl disulfides, 5-thiol-2-nitrobenzoic acid thiol (TNB-thiol), and the like. An aldehyde functional group can be coupled to amine- or hydrazide-containing molecules, and an azide group can react with a trivalent phosphorous group to form phosphoramidate or phosphorimide linkages. Suitable methods to introduce activating groups into molecules are known in the art (see for example, Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, Calif. (1996)). An activating group can be bonded directly to the organic group (e.g., hydrophilic polymer, fatty acid, fatty acid ester), or through a linker moiety, for example a divalent C_1–C_{12} group wherein one or more carbon atoms can be replaced by a heteroatom such as oxygen, nitrogen or sulfur. Suitable linker moieties include, for example, tetraethylene glycol, $—(CH_2)_3—$, $—NH—(CH_2)_6—NH—$, $—(CH_2)_2—NH—$ and $—CH_2—O—CH_2—CH_2—O—CH_2—CH_2—O—CH—NH—$. Modifying agents that comprise a linker moiety can be produced, for example, by reacting a mono-Boc-alkyldiamine (e.g., mono-Boc-ethylenediamine, mono-Boc-diaminohexane) with a fatty acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to form an amide bond between the free amine and the fatty acid carboxylate. The Boc protecting group can be removed from the product by treatment with trifluoroacetic acid (TFA) to expose a primary amine that can be coupled to another carboxylate as described, or can be reacted with maleic anhydride and the resulting product cyclized to produce an activated maleimido derivative of the fatty acid. (See, for example, Thompson, et al., WO 92/16221 the entire teachings of which are incorporated herein by reference.)

The modified antibodies of the invention can be produced by reacting a human antibody or antigen-binding fragment with a modifying agent. For example, the organic moieties can be bonded to the antibody in a non-site specific manner by employing an amine-reactive modifying agent, for example, an NHS ester of PEG. Modified human antibodies or antigen-binding fragments can also be prepared by reducing disulfide bonds (e.g., intra-chain disulfide bonds) of an antibody or antigen-binding fragment. The reduced antibody or antigen-binding fragment can then be reacted with a thiol-reactive modifying agent to produce the modified antibody of the invention. Modified human antibodies and antigen-binding fragments comprising an organic moiety that is bonded to specific sites of an antibody of the present invention can be prepared using suitable methods, such as reverse proteolysis (Fisch et al., *Bioconjugate Chem.*, 3:147–153 (1992); Werlen et al., *Bioconjugate Chem.*, 5:411–417 (1994); Kumaran et al., *Protein Sci.* 6(10): 2233–2241 (1997); Itoh et al., *Bioorg. Chem.*, 24(1): 59–68 (1996); Capellas et al., *Biotechnol. Bioeng.*, 56(4):456–463 (1997)), and the methods described in Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, Calif. (1996).

Anti-Idiotype Antibodies to Anti-IL-12 IG Derived Protein Compositions

In addition to monoclonal or chimeric anti-IL-12 antibodies, the present invention is also directed to an anti-idiotypic (anti-Id) antibody specific for such antibodies of the invention. An anti-Id antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding region of another antibody. The anti-Id can be prepared by immunizing an animal of the same species and genetic type (e.g. mouse strain) as the source of the Id antibody with the antibody or a CDR containing region thereof. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody and produce an anti-Id antibody. The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody.

Anti-IL-12 IG Derived Protein Compositions

The present invention also provides at least one anti-IL-12 antibody composition comprising at least one, at least two, at least three, at least four, at least five, at least six or more

anti-IL-12 antibodies thereof, as described herein and/or as known in the art that are provided in a non-naturally occurring composition, mixture or form. Such compositions comprise non-naturally occurring compositions comprising at least one or two full length, C- and/or N-terminally deleted variants, domains, fragments, or specified variants, of the anti-IL-12 antibody amino acid sequence selected from the group consisting of 70–100% of the contiguous amino acids of SEQ ID NOS:1, 2, 3, 4, 5, 6, 7 or 8, or specified fragments, domains or variants thereof. Preferred anti-IL-12 derived protein, fragment or variant compositions include at least one or two full length, fragments, domains or variants as at least one CDR containing portions of the anti-IL-12 antibody sequence of 70–100% of SEQ ID NOS: 1, 2, 3, 4, 5, 6, or specified fragments, domains or variants thereof. Further preferred compositions comprise 40–99% of at least one of 70–100% of SEQ ID NOS: 1, 2, 3, 4, 5, 6, or specified fragments, domains or variants thereof. Such composition percentages are by weight, volume, concentration, molarity, or molality as liquid or dry solutions, mixtures, suspension, emulsions or colloids, as known in the art or as described herein.

Anti-IL-12 antibody compositions of the present invention can further comprise at least one of any suitable and effective amount of a composition or pharmaceutical composition comprising at least one anti-IL-12 antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy, optionally further comprising at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalzine), a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAD)), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., an aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a fluororquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteriod, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoieitin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, domase alpha (Pulmozyme), a cytokine or a cytokine antagonist. Non-limiting examples of such cytokines include, but are not limited to, any of IL-1 to IL-23. Suitable dosages are well known in the art. See, e.g., Wells et al., eds., Pharmacotherapy Handbook, 2nd Edition, Appleton and Lange, Stamford, Conn. (2000); PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, Calif. (2000), each of which references are entirely incorporated herein by reference.

Such anti-cancer or anti-infectives can also include toxin molecules that are associated, bound, co-formulated or co-administered with at least one antibody of the present invention. The toxin can optionally act to selectively kill the pathologic cell or tissue. The pathologic cell can be a cancer or other cell. Such toxins can be, but are not limited to, purified or recombinant toxin or toxin fragment comprising at least one functional cytotoxic domain of toxin, e.g., selected from at least one of ricin, diphtheria toxin, a venom toxin, or a bacterial toxin. The term toxin also includes both endotoxins and exotoxins produced by any naturally occurring, mutant or recombinant bacteria or viruses which may cause any pathological condition in humans and other mammals, including toxin shock, which can result in death. Such toxins may include, but are not limited to, enterotoxigenic *E. coli* heat-labile enterotoxin (LT), heat-stable enterotoxin (ST), *Shigella* cytotoxin, *Aeromonas* enterotoxins, toxic shock syndrome toxin-1 (TSST-1), Staphylococcal enterotoxin A (SEA), B (SEB), or C (SEC), Streptococcal enterotoxins and the like. Such bacteria include, but are not limited to, strains of a species of enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (e.g., strains of serotype 0157:H7), *Staphylococcus* species (e.g., *Staphylococcus aureus, Staphylococcus pyogenes*), *Shigella* species (e.g., *Shigella dysenteriae, Shigella flexneri, Shigella boydii*, and *Shigella sonnei*), *Salmonella* species (e.g., *Salmonella typhi, Salmonella cholera-suis, Salmonella enteritidis*), *Clostridium* species (e.g., *Clostridium perfringens, Clostridium dificile, Clostridium botulinum*), *Camphlobacter* species (e.g., *Camphlobacter jejuni, Camphlobacter fetus*), *Heliobacter* species, (e.g., *Heliobacter pylori*), *Aeromonas* species (e.g., *Aeromonas sobria, Aeromonas hydrophila, Aeromonas caviae*), *Pleisomonas shigelloides, Yersina enterocolitica, Vibrios* species (e.g., *Vibrios cholerae, Vibrios parahemolyticus*), *Klebsiella* species, *Pseudomonas aeruginosa*, and *Streptococci*. See, e.g., Stein, ed., INTERNAL MEDICINE, 3rd ed., pp 1–13, Little, Brown and Co., Boston, (1990); Evans et al., eds., Bacterial Infections of Humans: Epidemiology and Control, 2d. Ed., pp 239–254, Plenum Medical Book Co., New York (1991); Mandell et al, Principles and Practice of Infectious Diseases, 3d. Ed., Churchill Livingstone, New York (1990); Berkow et al, eds., *The Merck Manual*, 16th edition, Merck and Co., Rahway, N.J., 1992; Wood et al, FEMS Microbiology Immunology, 76:121–134 (1991); Marrack et al, Science, 248:705–711 (1990), the contents of which references are incorporated entirely herein by reference.

Anti-IL-12 antibody compounds, compositions or combinations of the present invention can further comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. Pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but limited to, Gennaro, Ed., *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Co. (Easton, Pa.) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the anti-IL-12 antibody, fragment or variant composition as well known in the art or as described herein.

Pharmaceutical excipients and additives useful in the present composition include but are not limited to proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or

sugar polymers), which can be present singly or in combination, comprising alone or in combination 1–99.99% by weight or volume. Exemplary protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. One preferred amino acid is glycine.

Carbohydrate excipients suitable for use in the invention include, for example, monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. Preferred carbohydrate excipients for use in the present invention are mannitol, trehalose, and raffinose.

Anti-IL-12 antibody compositions can also include a buffer or a pH adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Preferred buffers for use in the present compositions are organic acid salts such as citrate.

Additionally, anti-IL-12 antibody compositions of the invention can include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrates (e.g., cyclodextrins, such as 2-hydroxypropyl-β-cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as "TWEEN 20" and "TWEEN 80"), lipids (e.g., phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA).

These and additional known pharmaceutical excipients and/or additives suitable for use in the anti-IL-12 antibody, portion or variant compositions according to the invention are known in the art, e.g., as listed in "Remington: The Science & Practice of Pharmacy", 19th ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52nd ed., Medical Economics, Montvale, N.J. (1998), the disclosures of which are entirely incorporated herein by reference. Preferred carrier or excipient materials are carbohydrates (e.g., saccharides and alditols) and buffers (e.g., citrate) or polymeric agents.

Formulations

As noted above, the invention provides for stable formulations, which is preferably a phosphate buffer with saline or a chosen salt, as well as preserved solutions and formulations containing a preservative as well as multi-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one anti-IL-12 antibody in a pharmaceutically acceptable formulation. Preserved formulations contain at least one known preservative or optionally selected from the group consisting of at least one phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (e.g., hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent. Any suitable concentration or mixture can be used as known in the art, such as 0.001–5%, or any range or value therein, such as, but not limited to 0.001, 0.003, 0.005, 0.009, 0.01, 0.02, 0.03, 0.05, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.3, 4.5, 4.6, 4.7, 4.8, 4.9, or any range or value therein. Non-limiting examples include, no preservative, 0.1–2% m-cresol (e.g., 0.2, 0.3, 0.4, 0.5, 0.9, 1.0%), 0.1–3% benzyl alcohol (e.g., 0.5, 0.9, 1.1., 1.5, 1.9, 2.0, 2.5%), 0.001–0.5% thimerosal (e.g., 0.005, 0.01), 0.001–2.0% phenol (e.g., 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005–1.0% alkylparaben(s) (e.g., 0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, 1.0%), and the like.

As noted above, the invention provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of at least one anti-IL-12 antibody with the prescribed buffers and/or preservatives, optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater. The invention further comprises an article of manufacture, comprising packaging material, a first vial comprising lyophilized at least one anti-IL-12 antibody, and a second vial comprising an aqueous diluent of prescribed buffer or preservative, wherein said packaging material comprises a label that instructs a patient to reconstitute the at least one anti-IL-12 antibody in the aqueous diluent to form a solution that can be held over a period of twenty-four hours or greater.

The at least one anti-IL-12 antibody used in accordance with the present invention can be produced by recombinant means, including from mammalian cell or transgenic preparations, or can be purified from other biological sources, as described herein or as known in the art.

The range of at least one anti-IL-12 antibody in the product of the present invention includes amounts yielding upon reconstitution, if in a wet/dry system, concentrations from about 1.0 μg/ml to about 1000 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, e.g., solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods.

Preferably, the aqueous diluent optionally further comprises a pharmaceutically acceptable preservative. Preferred preservatives include those selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof. The concentration of preservative used in the formulation is a concentration sufficient to yield an antimicrobial effect. Such concentrations are dependent on the preservative selected and are readily determined by the skilled artisan.

Other excipients, e.g. isotonicity agents, buffers, antioxidants, preservative enhancers, can be optionally and preferably added to the diluent. An isotonicity agent, such as glycerin, is commonly used at known concentrations. A physiologically tolerated buffer is preferably added to provide improved pH control. The formulations can cover a wide range of pHs, such as from about pH 4 to about pH 10, and preferred ranges from about pH 5 to about pH 9, and a most preferred range of about 6.0 to about 8.0. Preferably the formulations of the present invention have pH between about 6.8 and about 7.8. Preferred buffers include phosphate

buffers, most preferably sodium phosphate, particularly phosphate buffered saline (PBS).

Other additives, such as a pharmaceutically acceptable solubilizers like Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 40 (polyoxyethylene (20) sorbitan monopalmitate), Tween 80 (polyoxyethylene (20) sorbitan monooleate), Pluronic F68 (polyoxyethylene polyoxypropylene block copolymers), and PEG (polyethylene glycol) or non-ionic surfactants such as polysorbate 20 or 80 or poloxamer 184 or 188, Pluronic® polyls, other block co-polymers, and chelators such as EDTA and EGTA can optionally be added to the formulations or compositions to reduce aggregation. These additives are particularly useful if a pump or plastic container is used to administer the formulation. The presence of pharmaceutically acceptable surfactant mitigates the propensity for the protein to aggregate.

The formulations of the present invention can be prepared by a process which comprises mixing at least one anti-IL-12 antibody and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben, (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal or mixtures thereof in an aqueous diluent. Mixing the at least one anti-IL-12 antibody and preservative in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one anti-IL-12 antibody in buffered solution is combined with the desired preservative in a buffered solution in quantities sufficient to provide the protein and preservative at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

The claimed formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one anti-IL-12 antibody that is reconstituted with a second vial containing water, a preservative and/or excipients, preferably a phosphate buffer and/or saline and a chosen salt, in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus can provide a more convenient treatment regimen than currently available.

The present claimed articles of manufacture are useful for administration over a period of immediately to twenty-four hours or greater. Accordingly, the presently claimed articles of manufacture offer significant advantages to the patient. Formulations of the invention can optionally be safely stored at temperatures of from about 2 to about 40° C. and retain the biologically activity of the protein for extended periods of time, thus, allowing a package label indicating that the solution can be held and/or used over a period of 6, 12, 18, 24, 36, 48, 72, or 96 hours or greater. If preserved diluent is used, such label can include use up to 1–12 months, one-half, one and a half, and/or two years.

The solutions of at least one anti-IL-12 antibody in the invention can be prepared by a process that comprises mixing at least one antibody in an aqueous diluent. Mixing is carried out using conventional dissolution and mixing procedures. To prepare a suitable diluent, for example, a measured amount of at least one antibody in water or buffer is combined in quantities sufficient to provide the protein and optionally a preservative or buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

The claimed products can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one anti-IL-12 antibody that is reconstituted with a second vial containing the aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

The claimed products can be provided indirectly to patients by providing to pharmacies, clinics, or other such institutions and facilities, clear solutions or dual vials comprising a vial of lyophilized at least one anti-IL-12 antibody that is reconstituted with a second vial containing the aqueous diluent. The clear solution in this case can be up to one liter or even larger in size, providing a large reservoir from which smaller portions of the at least one antibody solution can be retrieved one or multiple times for transfer into smaller vials and provided by the pharmacy or clinic to their customers and/or patients.

Recognized devices comprising these single vial systems include those pen-injector devices for delivery of a solution such as BD Pens, BD Autojector®, Humaject®, NovoPen®, B-D®Pen, AutoPen®, and OptiPen®, GenotropinPen®, Genotronorm Pen®, Humatro Pen®, Reco-Pen®, Roferon Pen®, Biojector®, iject®, J-tip Needle-Free Injector®, Intraject®, Medi-Ject®, e.g., as made or developed by Becton Dickensen (Franklin Lakes, N.J., www.bectondickenson.com), Disetronic (Burgdorf, Switzerland, www.disetronic.com; Bioject, Portland, Oreg. (www.bioject.com); National Medical Products, Weston Medical (Peterborough, UK, www.weston-medical.com), Medi-Ject Corp (Minneapolis, Minn., www.mediject.com). Recognized devices comprising a dual vial system include those pen-injector systems for reconstituting a lyophilized drug in a cartridge for delivery of the reconstituted solution such as the HumatroPen®.

The products presently claimed include packaging material. The packaging material provides, in addition to the information required by the regulatory agencies, the conditions under which the product can be used. The packaging material of the present invention provides instructions to the patient to reconstitute the at least one anti-IL-12 antibody in the aqueous diluent to form a solution and to use the solution over a period of 2–24 hours or greater for the two vial, wet/dry, product. For the single vial, solution product, the label indicates that such solution can be used over a period of 2–24 hours or greater. The presently claimed products are useful for human pharmaceutical product use.

The formulations of the present invention can be prepared by a process that comprises mixing at least one anti-IL-12 antibody and a selected buffer, preferably a phosphate buffer containing saline or a chosen salt. Mixing the at least one antibody and buffer in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable formulation, for example, a measured amount of at least one antibody in water or buffer is combined with the desired buffering agent in water in quantities sufficient to provide the protein and buffer at the desired concentrations. Variations of this process would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the

temperature and pH at which the formulation is prepared, are all factors that can be optimized for the concentration and means of administration used.

The claimed stable or preserved formulations can be provided to patients as clear solutions or as dual vials comprising a vial of lyophilized at least one anti-IL-12 antibody that is reconstituted with a second vial containing a preservative or buffer and excipients in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of patient treatment and thus provides a more convenient treatment regimen than currently available.

At least one anti-IL-12 antibody in either the stable or preserved formulations or solutions described herein, can be administered to a patient in accordance with the present invention via a variety of delivery methods including SC or IM injection; transdermal, pulmonary, transmucosal, implant, osmotic pump, cartridge, micro pump, or other means appreciated by the skilled artisan, as well-known in the art.

Therapeutic Applications

The present invention also provides a method for modulating or treating at least one immune related disease, in a cell, tissue, organ, animal, or patient including, but not limited to, at least one of rheumatoid arthritis, juvenile rheumatoid arthritis, systemic onset juvenile theumatoid arthritis, psoriatic arthritis, ankylosing spondilitis, gastric ulcer, seronegative arthropathies, osteoarthritis, inflammatory bowel disease, ulcerative colitis, systemic lupus erythematosus, antiphospholipid syndrome, iridocyclitis/uveitis/optic neuritis, idiopathic pulmonary fibrosis, systemic vasculitis/wegener's granulomatosis, sarcoidosis, orchitis/vasectomy reversal procedures, allergic/atopic diseases, asthma, allergic rhinitis, eczema, allergic contact dermatitis, allergic conjunctivitis, hypersensitivity pneumonitis, transplants, organ transplant rejection, graft-versus-host disease, systemic inflammatory response syndrome, sepsis syndrome, gram positive sepsis, gram negative sepsis, culture negative sepsis, fungal sepsis, neutropenic fever, urosepsis, meningococcemia, trauma/hemorrhage, burns, ionizing radiation exposure, acute pancreatitis, adult respiratory distress syndrome, rheumatoid arthritis, alcohol-induced hepatitis, chronic inflammatory pathologies, sarcoidosis, Crohn's pathology, sickle cell anemia, diabetes, nephrosis, atopic diseases, hypersensitivity reactions, allergic rhinitis, hay fever, perennial rhinitis, conjunctivitis, endometriosis, asthma, urticaria, systemic anaphalaxis, dermatitis, pernicious anemia, hemolytic disease, thrombocytopenia, graft rejection of any organ or tissue, kidney transplant rejection, heart transplant rejection, liver transplant rejection, pancreas transplant rejection, lung transplant rejection, bone manow transplant (BMT) rejection, skin allograft rejection, cartilage transplant rejection, bone graft rejection, small bowel transplant rejection, fetal thymus implant rejection, parathyroid transplant rejection, xenograft rejection of any organ or tissue, allograft rejection, anti-receptor hypersensitivity reactions, Graves disease, Raynoud's disease, type B insulin-resistant diabetes, asthma, myasthenia gravis, antibody-meditated cytotoxicity, type III hypersensitivity reactions, systemic lupus erythematosus, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes syndrome, antiphospholipid syndrome, pemphigus, scleroderma, mixed connective tissue disease, idiopathic Addison's disease, diabetes mellitus, chronic active hepatitis, primary billiary cirrhosis, vitiligo, vasculitis, post-MI cardiotomy syndrome, type IV hypersensitivity, contact dermatitis, hypersensitivity pneumonitis, allograft rejection, granulomas due to intracellular organisms, drug sensitivity, metabolic/idiopathic, Wilson's disease, hemachromatosis, alpha-1-antitrypsin deficiency, diabetic retinopathy, hashimoto's thyroiditis, osteoporosis, hypothalamic-pituitary-adrenal axis evaluation, primary biliary cirrhosis, thyroiditis, encephalomyelitis, cachexia, cystic fibrosis, neonatal chronic lung disease, chronic obstructive pulmonary disease (COPD), familial hematophagocytic lymphohistiocytosis, dermatologic conditions, psoriasis, alopecia, nephrotic syndrome, nephritis, glomerular nephritis, acute renal failure, hemodialysis, uremia, toxicity, preeclampsia, okt3 therapy, anti-cd3 therapy, cytokine therapy, chemotherapy, radiation therapy (e.g., including but not limited to asthenia, anemia, cachexia, and the like), chronic salicylate intoxication, and the like. See, e.g., the Merck Manual, 12th–17th Editions, Merck & Company, Rahway, N.J. (1972, 1977, 1982, 1987, 1992, 1999), Pharmacotherapy Handbook, Wells et al., eds., Second Edition, Appleton and Lange, Stamford, Conn. (1998, 2000), each entirely incorporated by reference.

The present invention also provides a method for modulating or treating at least one cardiovascular disease in a cell, tissue, organ, animal, or patient, including, but not limited to, at least one of cardiac stun syndrome, myocardial infarction, congestive heart failure, stroke, ischemic stroke, hemorrhage, arteriosclerosis, atherosclerosis, restenosis, diabetic atherosclerotic disease, hypertension, arterial hypertension, renovascular hypertension, syncope, shock, syphilis of the cardiovascular system, heart failure, cor pulmonale, primary pulmonary hypertension, cardiac arrhythmias, atrial ectopic beats, atrial flutter, atrial fibrillation (sustained or paroxysmal), post perfusion syndrome, cardiopulmonary bypass inflammation response, chaotic or multifocal atrial tachycardia, regular narrow QRS tachycardia, specific arrythmias, ventricular fibrillation, His bundle arrythmias, atrioventricular block, bundle branch block, myocardial ischemic disorders, coronary artery disease, angina pectoris, myocardial infarction, cardiomyopathy, dilated congestive cardiomyopathy, restrictive cardiomyopathy, valvular heart diseases, endocarditis, pericardial disease, cardiac tumors, aortic and peripheral aneurysms, nortic dissection, inflammation of the aorta, occlusion of the abdominal aorta and its branches, peripheral vascular disorders, occlusive arterial disorders, peripheral atherosclerotic disease, thromboangitis obliterans, functional peripheral arterial disorders, Raynaud's phenomenon and disease, acrocyanosis, erythromelalgia, venous diseases, venous thrombosis, varicose veins, arteriovenous fistula, lymphederma, lipedema, unstable angina, reperfusion injury, post pump syndrome, ischemia-reperfusion injury, and the like. Such a method can optionally comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one anti-IL-12 antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy.

The present invention also provides a method for modulating or treating at least one infectious disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: acute or chronic bacterial infection, acute and chronic parasitic or infectious processes, including bacterial, viral and fungal infections, HIV infection/HIV neuropathy, meningitis, hepatitis (A, B or C, or the like), septic arthritis,

peritonitis, pneumonia, epiglottitis, *e. coli* 0157:h7, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, malaria, dengue hemorrhagic fever, leishmaniasis, leprosy, toxic shock syndrome, streptococcal myositis, gas gangrene, mycobacterium tuberculosis, mycobacterium avium intracellulare, pneumocystis carinii pneumonia, pelvic inflammatory disease, orchitis/epidydimitis, legionella, lyme disease, influenza a, epstein-barr virus, vital-associated hemaphagocytic syndrome, vital encephalitis/aseptic meningitis, and the like;

The present invention also provides a method for modulating or treating at least one malignant disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodyplastic syndrome (MDS), a lymphoma, Hodgkin's disease, a malignant lymphoma, non-hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, Kaposi's sarcoma, colorectal carcinoma, pancreatic carcinoma, nasopharyngeal carcinoma, malignant histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, solid tumors, adenocarcinomas, sarcomas, malignant melanoma, hemangioma, metastatic disease, cancer related bone resorption, cancer related bone pain, and the like.

The present invention also provides a method for modulating or treating at least one neurologic disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: neurodegenerative diseases, multiple sclerosis, migraine headache, AIDS dementia complex, demyelinating diseases, such as multiple sclerosis and acute transverse mycitis; extrapyramidal and cerebellar disorders, such as lesions of the corticospinal system; disorders of the basal ganglia or cerebellar disorders; hyperkinetic movement disorders, such as Huntington's Chorea and senile chorea; drug-induced movement disorders, such as those induced by drugs which block CNS dopamine receptors; hypokinetic movement disorders, such as Parkinson's disease; Progressive supranucleo Palsy; structural lesions of the cerebellum; spinocerebellar degenerations, such as spinal ataxia, Friedreich's ataxia, cerebellar cortical degenerations, multiple systems degenerations (Mencel, Dejerine-Thomas, Shi-Drager, and Machado-Joseph); systemic disorders (Refsum's disease, abetalipoprotemia, ataxia, telangiectasia, and mitochondirial multi-system disorder); demyelinating core disorders, such as multiple sclerosis, acute transverse myelitis; and disorders of the motor unit, such as neurogenic muscular atrophies (anterior horn cell degeneration, such as amyotrophic lateral sclerosis, infantile spinal muscular atrophy and juvenile spinal muscular atrophy); Alzheimer's disease; Down's Syndrome in middle age; Diffuse Lewy body disease; Senile Dementia of Lewy body type; Wernicke-Korsakoff syndrome; chronic alcoholism; Creutzfeldt-Jakob disease; Subacute sclerosing panencephalitis, Hallerrorden-Spatz disease; and Dementia pugilistica, and the like. Such a method can optionally comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one TNF antibody or specified portion or variant to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. See, e.g., the Merck Manual, 16th Edition, Merck & Company, Rahway, N.J. (1992).

Any method of the present invention can comprise administering an effective amount of a composition or pharmaceutical composition comprising at least one anti-IL-12 antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such immune diseases, wherein the administering of said at least one anti-IL-12 antibody, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalzine), a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a flurorquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteriod, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoietin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, domase alpha (Pulmozyme), a cytokine or a cytokine antagonist. Suitable dosages are well known in the art. See, e.g., Wells et al., eds., Pharmacotherapy Handbook, 2nd Edition, Appleton and Lange, Stamford, Conn. (2000); PDR Pharmacopoeia, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, Calif. (2000), each of which references are entirely incorporated herein by reference.

TNF antagonists suitable for compositions, combination therapy, co-administration, devices and/or methods of the present invention (further comprising at least one anti body, specified portion and variant thereof, of the present invention), include, but are not limited to, anti-TNF antibodies, antigen-binding fragments thereof, and receptor molecules which bind specifically to TNF; compounds which prevent and/or inhibit TNF synthesis, TNF release or its action on target cells, such as thalidomide, tenidap, phosphodiesterase inhibitors (e.g, pentoxifylline and rolipram), A2b adenosine receptor agonists and A2b adenosine receptor enhancers; compounds which prevent and/or inhibit TNF receptor signalling, such as mitogen activated protein (MAP) kinase inhibitors; compounds which block and/or inhibit membrane TNF cleavage, such as metalloproteinase inhibitors; compounds which block and/or inhibit TNF activity, such as angiotensin converting enzyme (ACE) inhibitors (e.g., captopril); and compounds which block and/or inhibit TNF production and/or synthesis, such as MAP kinase inhibitors.

As used herein, a "tumor necrosis factor antibody," "TNF antibody," "TNFα antibody," or fragment and the like decreases, blocks, inhibits, abrogates or interferes with

TNFα activity in vitro, in situ and/or preferably in vivo. For example, a suitable TNF human antibody of the present invention can bind TNFα and includes anti-TNF antibodies, antigen-binding fragments thereof, and specified mutants or domains thereof that bind specifically to TNFα. A suitable TNF antibody or fragment can also decrease block, abrogate, interfere, prevent and/or inhibit TNF RNA, DNA or protein synthesis, TNF release, TNF receptor signaling, membrane TNF cleavage, TNF activity, TNF production and/or synthesis.

Chimeric antibody cA2 consists of the antigen binding variable region of the high-affinity neutralizing mouse anti-human TNFα IgG1 antibody, designated A2, and the constant regions of a human IgG1, kappa immunoglobulin. The human IgG1 Fc region improves allogeneic antibody effector function, increases the circulating serum half-life and decreases the immunogenicity of the antibody. The avidity and epitope specificity of the chimeric antibody cA2 is derived from the variable region of the murine antibody A2. In a particular embodiment, a preferred source for nucleic acids encoding the variable region of the murine antibody A2 is the A2 hybridoma cell line.

Chimeric A2 (cA2) neutralizes the cytotoxic effect of both natural and recombinant human TNFα in a dose dependent manner. From binding assays of chimeric antibody cA2 and recombinant human TNFα, the affinity constant of chimeric antibody cA2 was calculated to be $1.04\times10^{10}M^{-1}$. Preferred methods for determining monoclonal antibody specificity and affinity by competitive inhibition can be found in Harlow, et al., *antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988; Colligan et al., eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, New York, (1992–2000); Kozbor et al., *Immunol. Today*, 4:72–79 (1983); Ausubel et al., eds. *Current Protocols in Molecular Biology*, Wiley Interscience, New York (1987–2000); and Muller, *Meth. Enzymol.*, 92:589–601 (1983), which references are entirely incorporated herein by reference.

In a particular embodiment, murine monoclonal antibody A2 is produced by a cell line designated c134A. Chimeric antibody cA2 is produced by a cell line designated c168A.

Additional examples of monoclonal anti-TNF antibodies that can be used in the present invention are described in the art (see, e.g., U.S. Pat. No. 5,231,024; Möller, A. et al., *Cytokine* 2(3):162–169 (1990); U.S. application Ser. No. 07/943,852 (filed Sep. 11, 1992); Rathjen et al., International Publication No. WO 91/02078 (published Feb. 21, 1991); Rubin et al., EPO Patent Publication No. 0 218 868 (published Apr. 22, 1987); Yone et al., EPO Patent Publication No. 0 288 088 (Oct. 26, 1988); Liang, et al., *Biochem. Biophys. Res. Comm.* 137:847–854 (1986); Meager, et al., *Hybridoma* 6:305–311 (1987); Fendly et al., *Hybridoma* 6:359–369 (1987); Bringman, et al., *Hybridoma* 6:489–507 (1987); and Hirai, et al., *J. Immunol. Meth.* 96:57–62 (1987), which references are entirely incorporated herein by reference).

TNF Receptor Molecules

Preferred TNF receptor molecules useful in the present invention are those that bind TNFα with high affinity (see, e.g., Feldmann et al., International Publication No. WO 92/07076 (published Apr. 30, 1992); Schall el al., *Cell* 61:361–370 (1990); and Loetscher et al., *Cell* 61:351–359 (1990), which references are entirely incorporated herein by reference) and optionally possess low immunogenicity. In particular, the 55 kDa (p55 TNF-R) and the 75 kDa (p75 TNF-R) TNF cell surface receptors are useful in the present invention. Truncated forms of these receptors, comprising the extracellular domains (ECD) of the receptors or functional portions thereof (see, e.g., Corcoran et al., *Eur. J. Biochem.* 223:831–844) (1994)), are also useful in the present invention. Truncated forms of the TNF receptors, comprising the ECD, have been detected in urine and serum as 30 kDa and 40 kDa TNFα inhibitory binding proteins (Engelmann, H. et al., *J. Biol. Chem.* 265:1531–1536 (1990)). TNF receptor multimeric molecules and TNF immunoreceptor fusion molecules, and derivatives and fragments or portions thereof, are additional examples of TNF receptor molecules which are useful in the methods and compositions of the present invention. The TNF receptor molecules which can be used in the invention are characterized by their ability to treat patients for extended periods with good to excellent alleviation of symptoms and low toxicity. Low immunogenicity and/or high affinity, as well as other undefined properties, can contribute to the therapeutic results achieved.

TNF receptor multimeric molecules useful in the present invention comprise all or a functional portion of the ECD of two or more TNF receptors linked via one or more polypeptide linkers or other nonpeptide linkers, such as polyethylene glycol (PEG). The multimeric molecules can further comprise a signal peptide of a secreted protein to direct expression of the multimeric molecule. These multimeric molecules and methods for their production have been described in U.S. application Ser. No. 08/437,533 (filed May 9, 1995), the content of which is entirely incorporated herein by reference.

TNF immunoreceptor fusion molecules useful in the methods and compositions of the present invention comprise at least one portion of one or more immunoglobulin molecules and all or a functional portion of one or more TNF receptors. These immunoreceptor fusion molecules can be assembled as monomers, or hetero- or homo-multimers. The immunoreceptor fusion molecules can also be monovalent or multivalent. An example of such a TNF immunoreceptor fusion molecule is TNF receptor/IgG fusion protein. TNF immunoreceptor fusion molecules and methods for their production have been described in the art (Lesslauer et al., *Eur. J. Immunol.* 21:2883–2886 (1991); Ashkenazi et al., *Proc. Natl. Acad. Sci. USA* 88:10535–10539 (1991); Peppel et al., *J. Exp. Med.* 174:1483–1489 (1991); Kolls et al., *Proc. Natl. Acad. Sci. USA* 91:215–219 (1994); Butler et al., *Cytokine* 6(6):616–623 (1994); Baker et al., *Eur. J. Immunol.* 24:2040–2048 (1994); Beutler et al., U.S. Pat. No. 5,447,851; and U.S. application Ser. No. 08/442,133 (filed May 16, 1995), each of which references are entirely incorporated herein by reference). Methods for producing immunoreceptor fusion molecules can also be found in Capon et al., U.S. Pat. No. 5,116,964; Capon et al., U.S. Pat. No. 5,225,538; and Capon et al., *Nature* 337:525–531 (1989), which references are entirely incorporated herein by reference.

A functional equivalent, derivative, fragment or region of TNF receptor molecule refers to the portion of the TNF receptor molecule, or the portion of the TNF receptor molecule sequence which encodes TNF receptor molecule, that is of sufficient size and sequences to functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNFα with high affinity and possess low immunogenicity). A functional equivalent of TNF receptor molecule also includes modified TNF receptor molecules that functionally resemble TNF receptor molecules that can be used in the present invention (e.g., bind TNFα with high affinity and possess low immunogenicity).

For example, a functional equivalent of TNF receptor molecule can contain a "SILENT" codon or one or more amino acid substitutions, deletions or additions (e.g., substitution of one acidic amino acid for another acidic amino acid; or substitution of one codon encoding the same or different hydrophobic amino acid for another codon encoding a hydrophobic amino acid). See Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Assoc. and Wiley-Interscience, New York (1987–2000).

Cytokines include any known cytokine. See, e.g., www.CopewithCytokines.com. Cytokine antagonists include, but are not limited to, any antibody, fragment or mimetic, any soluble receptor, fragment or mimetic, any small molecule antagonist, or any combination thereof.

Therapeutic Treatments.

Any method of the present invention can comprise a method for treating an IL-12 mediated disorder, comprising administering an effective amount of a composition or pharmaceutical composition comprising at least one anti-IL-12 antibody to a cell, tissue, organ, animal or patient in need of such modulation, treatment or therapy. Such a method can optionally further comprise co-administration or combination therapy for treating such immune diseases, wherein the administering of said at least one anti-IL-12 antibody, specified portion or variant thereof, further comprises administering, before concurrently, and/or after, at least one selected from at least one TNF antagonist (e.g., but not limited to a TNF antibody or fragment, a soluble TNF receptor or fragment, fusion proteins thereof, or a small molecule TNF antagonist), an antirheumatic (e.g., methotrexate, auranofin, aurothioglucose, azathioprine, etanercept, gold sodium thiomalate, hydroxychloroquine sulfate, leflunomide, sulfasalzine), a muscle relaxant, a narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial (e.g., aminoglycoside, an antifungal, an antiparasitic, an antiviral, a carbapenem, cephalosporin, a fluoroquinolone, a macrolide, a penicillin, a sulfonamide, a tetracycline, another antimicrobial), an antipsoriatic, a corticosteriod, an anabolic steroid, a diabetes related agent, a mineral, a nutritional, a thyroid agent, a vitamin, a calcium related hormone, an antidiarrheal, an antitussive, an antiemetic, an antiulcer, a laxative, an anticoagulant, an erythropoieitin (e.g., epoetin alpha), a filgrastim (e.g., G-CSF, Neupogen), a sargramostim (GM-CSF, Leukine), an immunization, an immunoglobulin, an immunosuppressive (e.g., basiliximab, cyclosporine, daclizumab), a growth hormone, a hormone replacement drug, an estrogen receptor modulator, a mydriatic, a cycloplegic, an alkylating agent, an antimetabolite, a mitotic inhibitor, a radiopharmaceutical, an antidepressant, antimanic agent, an antipsychotic, an anxiolytic, a hypnotic, a sympathomimetic, a stimulant, donepezil, tacrine, an asthma medication, a beta agonist, an inhaled steroid, a leukotriene inhibitor, a methylxanthine, a cromolyn, an epinephrine or analog, dornase alpha (Pulmozyme), a cytokine or a cytokine antagonist.

Typically, treatment of pathologic conditions is effected by administering an effective amount or dosage of at least one anti-IL-12 antibody composition that total, on average, a range from at least about 0.01 to 500 milligrams of at least one anti-IL-12 antibody per kilogram of patient per dose, and preferably from at least about 0.1 to 100 milligrams antibody/kilogram of patient per single or multiple administration, depending upon the specific activity of contained in the composition. Alternatively, the effective serum concentration can comprise 0.1–5000 μg/ml serum concentration per single or multiple administration. Suitable dosages are known to medical practitioners and will, of course, depend upon the particular disease state, specific activity of the composition being administered, and the particular patient undergoing treatment. In some instances, to achieve the desired therapeutic amount, it can be necessary to provide for repeated administration, i.e., repeated individual administrations of a particular monitored or metered dose, where the individual administrations are repeated until the desired daily dose or effect is achieved.

Preferred doses can optionally include 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 and/or 100–500 mg/kg/administration, or any range, value or fraction thereof, or to achieve a serum concentration of 0.1, 0.5, 0.9, 1.0, 1.1, 1.2, 1.5, 1.9, 2.0, 2.5, 2.9, 3.0, 3.5, 3.9, 4.0, 4.5, 4.9, 5.0, 5.5, 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11, 11.5, 11.9, 20, 12.5, 12.9, 13.0, 13.5, 13.9, 14.0, 14.5, 4.9, 5.0, 5.5., 5.9, 6.0, 6.5, 6.9, 7.0, 7.5, 7.9, 8.0, 8.5, 8.9, 9.0, 9.5, 9.9, 10, 10.5, 10.9, 11, 11.5, 11.9, 12, 12.5, 12.9, 13.0, 13.5, 13.9, 14, 14.5, 15, 15.5, 15.9, 16, 16.5, 16.9, 17, 17.5, 17.9, 18, 18.5, 18.9, 19, 19.5, 19.9, 20, 20.5, 20.9, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 96, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, and/or 5000 μg/ml serum concentration per single or multiple administration, or any range, value or fraction thereof.

Alternatively, the dosage administered can vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent, and its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms, kind of concurrent treatment, frequency of treatment, and the effect desired. Usually a dosage of active ingredient can be about 0.1 to 100 milligrams per kilogram of body weight. Ordinarily 0.1 to 50, and preferably 0.1 to 10 milligrams per kilogram per administration or in sustained release form is effective to obtain desired results.

As a non-limiting example, treatment of humans or animals can be provided as a one-time or periodic dosage of at least one antibody of the present invention 0.1 to 100 mg/kg, such as 0.5, 0.9, 1.0, 1.1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg, per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively or additionally, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, or 52, or alternatively or additionally, at least one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 years, or any combination thereof, using single, infusion or repeated doses.

Dosage forms (composition) suitable for internal administration generally contain from about 0.1 milligram to about 500 milligrams of active ingredient per unit or container. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5–99.999% by weight based on the total weight of the composition.

For parenteral administration, the antibody can be formulated as a solution, suspension, emulsion or lyophilized

powder in association, or separately provided, with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 1–10% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can contain additives that maintain isotonicity (e.g., sodium chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by known or suitable techniques.

Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

Alternative Administration

Many known and developed modes can be used according to the present invention for administering pharmaceutically effective amounts of at least one anti-IL-12 antibody according to the present invention. While pulmonary administration is used in the following description, other modes of administration can be used according to the present invention with suitable results.

IL-12 antibodies of the present invention can be delivered in a carrier, as a solution, emulsion, colloid, or suspension, or as a dry powder, using any of a variety of devices and methods suitable for administration by inhalation or other modes described here within or known in the art.

Parenteral Formulations and Administration

Formulations for parenteral administration can contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Aqueous or oily suspensions for injection can be prepared by using an appropriate emulsifier or humidifier and a suspending agent, according to known methods. Agents for injection can be a non-toxic, non-orally administrable diluting agent, such as an aqueous solution or a sterile injectable solution or suspension in a solvent. As the usable vehicle or solvent, water, Ringer's solution, isotonic saline, etc. are allowed; as an ordinary solvent, or suspending solvent, sterile involatile oil can be used. For these purposes, any kind of involatile oil and fatty acid can be used, including natural or synthetic or semisynthetic fatty oils or fatty acids; natural or synthetic or semisynthetic mono- or di- or tri-glycerides. Parental administration is known in the art and includes, but is not limited to, conventional means of injections, a gas pressured needle-less injection device as described in U.S. Pat. No. 5,851,198, and a laser perforator device as described in U.S. Pat. No. 5,839,446 entirely incorporated herein by reference.

Alternative Delivery

The invention further relates to the administration of at least one anti-IL-12 antibody by parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, or transdermal means. At least one anti-IL-12 antibody composition can be prepared for use for parenteral (subcutaneous, intramuscular or intravenous) or any other administration particularly in the form of liquid solutions or suspensions; for use in vaginal or rectal administration particularly in semisolid forms such as, but not limited to, creams and suppositories; for buccal, or sublingual administration such as, but not limited to, in the form of tablets or capsules; or intranasally such as, but not limited to, the form of powders, nasal drops or aerosols or certain agents; or transdermally such as not limited to a gel, ointment, lotion, suspension or patch delivery system with chemical enhancers such as dimethyl sulfoxide to either modify the skin structure or to increase the drug concentration in the transdermal patch (Junginger, et al. In "Drug Permeation Enhancement"; Hsieh, D. S., Eds., pp. 59–90 (Marcel Dekker, Inc. New York 1994, entirely incorporated herein by reference), or with oxidizing agents that enable the application of formulations containing proteins and peptides onto the skin (WO 98/53847), or applications of electric fields to create transient transport pathways such as electroporation, or to increase the mobility of charged drugs through the skin such as iontophoresis, or application of ultrasound such as sonophoresis (U.S. Pat. Nos. 4,309,989 and 4,767,402) (the above publications and patents being entirely incorporated herein by reference).

Pulmonary/Nasal Administration

For pulmonary administration, preferably at least one anti-IL-12 antibody composition is delivered in a particle size effective for reaching the lower airways of the lung or sinuses. According to the invention, at least one anti-IL-12 antibody can be delivered by any of a variety of inhalation or nasal devices known in the art for administration of a therapeutic agent by inhalation. These devices capable of depositing aerosolized formulations in the sinus cavity or alveoli of a patient include metered dose inhalers, nebulizers, dry powder generators, sprayers, and the like. Other devices suitable for directing the pulmonary or nasal administration of antibodies are also known in the art. All such devices can use of formulations suitable for the administration for the dispensing of antibody in an aerosol. Such aerosols can be comprised of either solutions (both aqueous and non aqueous) or solid particles. Metered dose inhalers like the Ventolin® metered dose inhaler, typically use a propellent gas and require actuation during inspiration (See, e.g., WO 94/16970, WO 98/35888). Dry powder inhalers like Turbuhaler™ (Astra), Rotahaler® (Glaxo), Diskus® (Glaxo), Spiros™ inhaler (Dura), devices marketed by Inhale Therapeutics, and the Spinhaler® powder inhaler (Fisons), use breath-actuation of a mixed powder (U.S. Pat. No. 4,668,218 Astra, EP 237507 Astra, WQ 97/25086 Glaxo, WO 94/08552 Dura, U.S. Pat. No. 5,458,135 Inhale, WO 94/06498 Fisons, entirely incorporated herein by reference). Nebulizers like AERx™ Aradigm, the Utravent® nebulizer (Mallinckrodt), and the Acorn II® nebulizer (Marquest Medical Products) (U.S. Pat. No. 5,404,871 Aradigm, WO 97/22376), the above references entirely incorporated herein by reference, produce aerosols from solutions, while metered dose inhalers, dry powder inhalers, etc. generate small particle aerosols. These specific examples of commercially available inhalation devices are intended to be a representative of specific devices suitable for the practice of this invention, and are not intended as limiting the scope of the invention. Preferably, a composition comprising at least one anti-IL-12 antibody is delivered by a dry powder inhaler or a sprayer. There are several desirable features of an inhalation device for administering at least one antibody of the present invention. For example, delivery by the inhalation device is advantageously reliable, reproducible, and accurate. The inhalation device can optionally deliver small dry particles, e.g. less than about 10 μm, preferably about 1–5 μm, for good respirability.

Administration of IL-12 antibody Compositions as a Spray

A spray including IL-12 antibody composition protein can be produced by forcing a suspension or solution of at least

one anti-IL-12 antibody through a nozzle under pressure. The nozzle size and configuration, the applied pressure, and the liquid feed rate can be chosen to achieve the desired output and particle size. An electrospray can be produced, for example, by an electric field in connection with a capillary or nozzle feed. Advantageously, particles of at least one anti-IL-12 antibody composition protein delivered by a sprayer have a particle size less than about 10 μm, preferably in the range of about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm.

Formulations of at least one anti-IL-12 antibody composition protein suitable for use with a sprayer typically include antibody composition protein in an aqueous solution at a concentration of about 0.1 mg to about 100 mg of at least one anti-IL-12 antibody composition protein per ml of solution or mg/gm, or any range or value therein, e.g., but not limited to, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/ml or mg/gm. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the antibody composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating antibody composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating antibody composition proteins include sucrose, mannitol, lactose, trehalose, glucose, or the like. The antibody composition protein formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the antibody composition protein caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 14% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan monooleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as IL-12 antibodies, or specified portions or variants, can also be included in the formulation.

Administration of IL-12 Antibody Compositions by a Nebulizer

Antibody composition protein can be administered by a nebulizer, such as jet nebulizer or an ultrasonic nebulizer. Typically, in a jet nebulizer, a compressed air source is used to create a high-velocity air jet through an orifice. As the gas expands beyond the nozzle, a low-pressure region is created, which draws a solution of antibody composition protein through a capillary tube connected to a liquid reservoir. The liquid stream from the capillary tube is sheared into unstable filaments and droplets as it exits the tube, creating the aerosol. A range of configurations, flow rates, and baffle types can be employed to achieve the desired performance characteristics from a given jet nebulizer. In an ultrasonic nebulizer, high-frequency electrical energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the formulation of antibody composition protein either directly or through a coupling fluid, creating an aerosol including the antibody composition protein. Advantageously, particles of antibody composition protein delivered by a nebulizer have a particle size less than about 10 μm, preferably in the range of about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm.

Formulations of at least one anti-IL-12 antibody suitable for use with a nebulizer, either jet or ultrasonic, typically include a concentration of about 0.1 mg to about 100 mg of at least one anti-IL-12 antibody protein per ml of solution. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the at least one anti-IL-12 antibody composition protein, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating at least one anti-IL-12 antibody composition proteins include albumin, protamine, or the like. Typical carbohydrates useful in formulating at least one anti-IL-12 antibody include sucrose, mannitol, lactose, trehalose, glucose, or the like. The at least one anti-IL-12 antibody formulation can also include a surfactant, which can reduce or prevent surface-induced aggregation of the at least one anti-IL-12 antibody caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbital fatty acid esters. Amounts will generally range between 0.001 and 4% by weight of the formulation. Especially preferred surfactants for purposes of this invention are polyoxyethylene sorbitan mono-oleate, polysorbate 80, polysorbate 20, or the like. Additional agents known in the art for formulation of a protein such as antibody protein can also be included in the formulation.

Administration of IL-12 Antibody Compositions by a Metered Dose Inhaler

In a metered dose inhaler (MDI), a propellant, at least one anti-IL-12 antibody, and any excipients or other additives are contained in a canister as a mixture including a liquefied compressed gas. Actuation of the metering valve releases the mixture as an aerosol, preferably containing particles in the size range of less than about 10 μm, preferably about 1 μm to about 5 μm, and most preferably about 2 μm to about 3 μm. The desired aerosol particle size can be obtained by employing a formulation of antibody composition protein produced by various methods known to those of skill in the art, including jet-milling, spray drying, critical point condensation, or the like. Preferred metered dose inhalers include those manufactured by 3M or Glaxo and employing a hydrofluorocarbon propellant.

Formulations of at least one anti-IL-12 antibody for use with a metered-dose inhaler device will generally include a finely divided powder containing at least one anti-IL-12 antibody as a suspension in a non-aqueous medium, for example, suspended in a propellant with the aid of a surfactant. The propellant can be any conventional material employed for this purpose, such as chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol and 1,1,1,2-tetrafluoroethane, HFA-134a (hydrofluroalkane-134a), HFA-227 (hydrofluroalkane-227), or the like. Preferably the propellant is a hydrofluorocarbon. The surfactant can be chosen to stabilize the at least one anti-IL-12 antibody as a suspension in the propellant, to protect the active agent against chemical degradation, and the like. Suitable surfactants include sorbitan trioleate, soya lecithin, oleic acid, or the like. In some cases solution aerosols are preferred using solvents such as ethanol. Additional agents known in the art for formulation of a protein such as protein can also be included in the formulation.

One of ordinary skill in the art will recognize that the methods of the current invention can be achieved by pul-

monary administration of at least one anti-IL-12 antibody compositions via devices not described herein.

Oral Formulations and Administration

Formulations for oral rely on the co-administration of adjuvants (e.g., resorcinols and nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to increase artificially the permeability of the intestinal walls, as well as the co-administration of enzymatic inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and trasylol) to inhibit enzymatic degradation. The active constituent compound of the solid-type dosage form for oral administration can be mixed with at least one additive, including sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, arginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, and glyceride. These dosage forms can also contain other type(s) of additives, e.g., inactive diluting agent, lubricant such as magnesium stearate, paraben, preserving agent such as sorbic acid, ascorbic acid, .alpha.-tocopherol, antioxidant such as cysteine, disintegrator, binder, thickener, buffering agent, sweetening agent, flavoring agent, perfuming agent, etc.

Tablets and pills can be further processed into enteric-coated preparations. The liquid preparations for oral administration include emulsion, syrup, elixir, suspension and solution preparations allowable for medical use. These preparations can contain inactive diluting agents ordinarily used in said field, e.g., water. Liposomes have also been described as drug delivery systems for insulin and heparin (U.S. Pat. No. 4,239,754). More recently, microspheres of artificial polymers of mixed amino acids (proteinoids) have been used to deliver pharmaceuticals (U.S. Pat. No. 4,925,673). Furthermore, carrier compounds described in U.S. Pat. No. 5,879,681 and U.S. Pat. No. 5,5,871,753 are used to deliver biologically active agents orally are known in the art.

Mucosal Formulations and Administration

For absorption through mucosal surfaces, compositions and methods of administering at least one anti-IL-12 antibody include an emulsion comprising a plurality of submicron particles, a mucoadhesive macromolecule, a bioactive peptide, and an aqueous continuous phase, which promotes absorption through mucosal surfaces by achieving mucoadhesion of the emulsion particles (U.S. Pat. Nos. 5,514,670). Mucous surfaces suitable for application of the emulsions of the present invention can include corneal, conjunctival, buccal, sublingual, nasal, vaginal, pulmonary, stomachic, intestinal, and rectal routes of administration. Formulations for vaginal or rectal administration, e.g. suppositories, can contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for intranasal administration can be solid and contain as excipients, for example, lactose or can be aqueous or oily solutions of nasal drops. For buccal administration excipients include sugars, calcium stearate, magnesium stearate, pregelinatined starch, and the like (U.S. Pat. No. 5,849,695).

Transdermal Formulations and Administration

For transdermal administration, the at least one anti-IL-12 antibody is encapsulated in a delivery device such as a liposome or polymeric nanoparticles, microparticle, microcapsule, or microspheres (referred to collectively as microparticles unless otherwise stated). A number of suitable devices are known, including microparticles made of synthetic polymers such as polyhydroxy acids such as polylactic acid, polyglycolic acid and copolymers thereof, polyorthoesters, polyanhydrides, and polyphosphazenes, and natural polymers such as collagen, polyamino acids, albumin and other proteins, alginate and other polysaccharides, and combinations thereof (U.S. Pat. No. 5,814,599).

Prolonged Administration and Formulations

It can be sometimes desirable to deliver the compounds of the present invention to the subject over prolonged periods of time, for example, for periods of one week to one year from a single administration. Various slow release, depot or implant dosage forms can be utilized. For example, a dosage form can contain a pharmaceutically acceptable non-toxic salt of the compounds that has a low degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid such as phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a salt with a polyvalent metal cation such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from e.g., N,N'-dibenzyl-ethylenediamine or ethylenediamine; or (c) combinations of (a) and (b) e.g. a zinc tannate salt. Additionally, the compounds of the present invention or, preferably, a relatively insoluble salt such as those just described, can be formulated in a gel, for example, an aluminum monostearate gel with, e.g. sesame oil, suitable for injection. Particularly preferred salts are zinc salts, zinc tannate salts, pamoate salts, and the like. Another type of slow release depot formulation for injection would contain the compound or salt dispersed for encapsulated in a slow degrading, non-toxic, non-antigenic polymer such as a polylactic acid/polyglycolic acid polymer for example as described in U.S. Pat. No. 3,773,919. The compounds or, preferably, relatively insoluble salts such as those described above can also be formulated in cholesterol matrix silastic pellets, particularly for use in animals. Additional slow release, depot or implant formulations, e.g. gas or liquid liposomes are known in the literature (U.S. Pat. No. 5,770,222 and "Sustained and Controlled Release Drug Delivery Systems", J. R. Robinson ed., Marcel Dekker, Inc., N.Y., 1978).

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

EXAMPLE 1

Cloning and Expression of IL-12 Antibody in Mammalian Cells

A typical mammalian expression vector contains at least one promoter element, which mediates the initiation of transcription of mRNA, the antibody coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription can be achieved with the early and late promoters from SV40, the long terminal repeats (LTRS) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter). Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pIRES1neo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clonetech Labs, Palo Alto, Calif.), pcDNA3.1 (+/−), pcDNA/Zeo (+/−) or pcDNA3.1/Hygro (+/−) (Invitrogen), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC

37146) and pBC12MI (ATCC 67109). Mammalian host cells that could be used include human Hela 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the gene can be expressed in stable cell lines that contain the gene integrated into a chromosome. The co-transfection with a selectable marker such as dhfr, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded antibody. The DHFR (dihydrofolate reductase) marker is useful to develop cell lines that carry several hundred or even several thousand copies of the gene of interest. Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy, et al., Biochem. J. 227:277-279 (1991); Bebbington, et al., Bio/Technology 10:169-175 (1992)). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of antibodies.

The expression vectors pC1 and pC4 contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen, et al., Molec. Cell. Biol. 5:438-447 (1985)) plus a fragment of the CMV-enhancer (Boshart, et al., Cell 41:521-530 (1985)). Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors contain in addition the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene.

Cloning and Expression in CHO Cells

The vector pC4 is used for the expression of IL-12 antibody. Plasmid pC4 is a derivative of the plasmid pSV2-dhfr (ATCC Accession No. 37146). The plasmid contains the mouse DHFR gene under control of the SV40 early promoter. Chinese hamster ovary- or other cells lacking dihydrofolate activity that are transfected with these plasmids can be selected by growing the cells in a selective medium (e.g., alpha minus MEM, Life Technologies, Gaithersburg, Md.) supplemented with the chemotherapeutic agent methotrexate. The amplification of the DHFR genes in cells resistant to methotrexate (MTX) has been well documented (see, e.g., F. W. Alt, et al., J. Biol. Chem. 253:1357-1370 (1978); J. L. Hamlin and C. Ma, Biochem. et Biophys. Acta 1097:107-143 (1990); and M. J. Page and M. A. Sydenham, Biotechnology 9:64-68 (1991)). Cells grown in increasing concentrations of MTX develop resistance to the drug by overproducing the target enzyme, DHFR, as a result of amplification of the DHFR gene. If a second gene is linked to the DHFR gene, it is usually co-amplified and over-expressed. It is known in the art that this approach can be used to develop cell lines carrying more than 1,000 copies of the amplified gene(s). Subsequently, when the methotrexate is withdrawn, cell lines are obtained that contain the amplified gene integrated into one or more chromosome(s) of the host cell.

Plasmid pC4 contains for expressing the gene of interest the strong promoter of the long terminal repeat (LTR) of the Rous Sarcoma Virus (Cullen, et al., Molec. Cell. Biol. 5:438-447 (1985)) plus a fragment isolated from the enhancer of the immediate early gene of human cytomegalovirus (CMV) (Boshart, et al., Cell 41:521-530 (1985)). Downstream of the promoter are BamHI, XbaI, and Asp718 restriction enzyme cleavage sites that allow integration of the genes. Behind these cloning sites the plasmid contains the 3' intron and polyadenylation site of the rat preproinsulin gene. Other high efficiency promoters can also be used for the expression, e.g., the human b-actin promoter, the SV40 early or late promoters or the long terminal repeats from other retroviruses, e.g., HIV and HTLVI. Clontech's Tet-Off and Tet-On gene expression systems and similar systems can be used to express the IL-12 in a regulated way in mammalian cells (M. Gossen, and H. Bujard, Proc. Natl. Acad. Sci. USA 89: 5547-5551 (1992)). For the polyadenylation of the mRNA other signals, e.g., from the human growth hormone or globin genes can be used as well. Stable cell lines carrying a gene of interest integrated into the chromosomes can also be selected upon co-transfection with a selectable marker such as gpt, G418 or hygromycin. It is advantageous to use more than one selectable marker in the beginning, e.g., G418 plus methotrexate.

The plasmid pC4 is digested with restriction enzymes and then dephosphorylated using calf intestinal phosphatase by procedures known in the art. The vector is then isolated from a 1% agarose gel.

The DNA sequence encoding the complete IL-12 antibody is used, e.g., as presented in SEQ ID NOS:1 and 2, corresponding to HC and LC variable regions of an IL-12 antibody of the present invention, according to known method steps. Isolated nucleic acid encoding a suitable human constant region (i.e., HC and LC regions) is also used in this construct (e.g., as provided in vector p1351).

The isolated variable and constant region encoding DNA and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB 101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary (CHO) cells lacking an active DHFR gene are used for transfection. 5 μg of the expression plasmid pC4 is cotransfected with 0.5 μg of the plasmid pSV2-neo using lipofecrin. The plasmid pSV2neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics, including G418. The cells are seeded in alpha minus MEM supplemented with 1 μg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 μg/ml G418. After about 10-14 days, single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrex ate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 mM, 2 mM, 5 mM, 10 mM, 20 mM). The same procedure is repeated until clones are obtained that grow at a concentration of 100-200 mM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reverse phase HPLC analysis.

EXAMPLE 2

Generation of High Affinity Human IgG Monoclonal Antibodies Reactive with Human IL-12 Using Transgenic Mice

SUMMARY

Transgenic mice have been used that contain human heavy and light chain immunoglobulin genes to generate high affinity, completely human, monoclonal antibodies that

can be used therapeutically to inhibit the action of IL-12 for the treatment of one or more IL-12-mediated disease. (CBA/J×C57/BL6/J) F_2 hybrid mice containing human variable and constant region antibody transgenes for both heavy and light chains are immunized with human recombinant IL-12 (Taylor et al., Intl. Immunol. 6:579–591 (1993); Lonberg, et al., Nature 368:856–859 (1994); Neuberger, M., Nature Biotech. 14:826 (1996); Fishwild, et al., Nature Biotechnology 14:845–851(1996)). Several fusions yielded one or more panels of completely human IL-12 reactive IgG monoclonal antibodies. The completely human anti-IL-12 antibodies are further characterized. All are IgG1κ. Such antibodies are found to have affinity constants somewhere between 1×10^9 and 9×10^{12}. The unexpectedly high affinities of these fully human monoclonal antibodies make them suitable candidates for therapeutic applications in IL-12 related diseases, pathologies or disorders.

Abbreviations

BSA—bovine serum albumin
CO_2—carbon dioxide
DMSO—dimethyl sulfoxide
EIA—enzyme immunoassay
FBS—fetal bovine serum
H_2O_2—hydrogen peroxide
HRP—horseradish peroxidase\
ID—interadermal
Ig—immunoglobulin
IL-12—interleukin-12
IP—intraperitoneal
IV—intravenous
Mab—monoclonal antibody
OD—optical density
OPD—o-Phenylenediamine dihydrochloride
PEG—polyethylene glycol
PSA—penicillin, streptomycin, amphotericin
RT—room temperature
SQ—subcutaneous
v/v—volume per volume
w/v—weight per volume

Materials and Methods

Animals

Transgenic mice that can express human antibodies are known in the art (and are commercially available (e.g., from GenPharm International, San Jose, Calif.; Abgenix, Freemont, Calif., and others) that express human immunoglobulins but not mouse IgM or Igκ. For example, such transgenic mice contain human sequence transgenes that undergo V(D)J joining, heavy-chain class switching, and somatic mutation to generate a repertoire of human sequence immunoglobulins (Lonberg, et al., Nature 368:856–859 (1994)). The light chain transgene can be derived, e.g., in part from a yeast artificial chromosome clone that includes nearly half of the germline human Vκ region. In addition, the heavy-chain transgene can encode both human and human γ1 (Fishwild, et al., Nature Biotechnology 14:845–851 (1996)) and/or γ3 constant regions. Mice derived from appropriate genotopic lineages can be used in the immunization and fusion processes to generate fully human monoclonal antibodies to IL-12.

Immunization

One or more immunization schedules can be used to generate the anti-IL-12 human hybridomas. The first several fusions can be performed after the following exemplary immunization protocol, but other similar known protocols can be used. Several 14–20 week old female and/or surgically castrated transgenic male mice are immunized IP and/or ID with 1–1000 μg of recombinant human IL-12 emulsified with an equal volume of TITERMAX or complete Freund's adjuvant in a final volume of 100–400 μL (e.g., 200). Each mouse can also optionally receive 1–10 μg in 100 μL physiological saline at each of 2 SQ sites. The mice can then be immunized 1–7, 5–12, 10–18, 17–25 and/or 21–34 days later IP (1–400 μg) and SQ (1–400 μg×2) with IL-12 emulsified with an equal volume of TITERMAX or incomplete Freund's adjuvant. Mice can be bled 12–25 and 25–40 days later by retro-orbital puncture without anti-coagulant. The blood is then allowed to clot at RT for one hour and the serum is collected and titered using an IL-12 EIA assay according to known methods. Fusions are performed when repeated injections do not cause titers to increase. At that time, the mice can be given a final IV booster injection of 1–400 μg IL-12 diluted in 100 μL physiological saline. Three days later, the mice can be euthanized by cervical dislocation and the spleens removed aseptically and immersed in 10 mL of cold phosphate buffered saline (PBS) containing 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (PSA). The splenocytes are harvested by sterilely perfusing the spleen with PSA-PBS. The cells are washed once in cold PSA-PBS, counted using Trypan blue dye exclusion and resuspended in RPMI 1640 media containing 25 mM Hepes.

Cell Fusion

Fusion can be carried out at a 1:1 to 1:10 ratio of murine myeloma cells to viable spleen cells according to known methods, e.g., as known in the art. As a non-limiting example, spleen cells and mycloma cells can be pelleted together. The pellet can then be slowly resuspended, over 30 seconds, in 1 mL of 50% (w/v) PEG/PBS solution (PEG molecular weight 1,450, Sigma) at 37° C. The fusion can then be stopped by slowly adding 10.5 mL of RPMI 1640 medium containing 25 mM Hepes (37° C.) over 1 minute. The fused cells are centrifuged for 5 minutes at 500–1500 rpm. The cells are then resuspended in HAT medium (RPMI 1640 medium containing 25 mM Hepes, 10% Fetal Clone I serum (Hyclone), 1 mM sodium pyruvate, 4 mM L-glutamine, 10 μg/mL gentamicin, 2.5% Origen culturing supplement (Fisher), 10% 653-conditioned RPMI 1640/Hepes media, 50 μM 2-mercaptoethanol, 100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine) and then plated at 200 μL/well in fifteen 96-well flat bottom tissue culture plates. The plates are then placed in a humidified 37° C. incubator containing 5% CO_2 and 95% air for 7–10 days.

Detection of Human IgG Anti-IL-12 Antibodies in Mouse Serum

Solid phase ETA's can be used to screen mouse sera for human IgG antibodies specific for human IL-12. Briefly, plates can be coated with IL-12 at 2 μg/mL in PBS overnight. After washing in 0.15M saline containing 0.02% (v/v) Tween 20, the wells can be blocked with 1% (w/v) BSA in PBS, 200 μL/well for 1 hour at RT. Plates are used immediately or frozen at −20° C. for future use. Mouse serum dilutions are incubated on the IL-12 coated plates at 50 μL/well at RT for 1 hour. The plates are washed and then probed with 50 μL/well HRP-labeled goat anti-human IgG, Fc specific diluted 1:30,000 in 1% BSA-PBS for 1 hour at RT. The plates can again be washed and 100 μL/well of the citrate-phosphate substrate solution (0.1M citric acid and 0.2M sodium phosphate, 0.01% H_2O_2 and 1 mg/mL OPD) is added for 15 minutes at RT. Stop solution (4N sulfuric acid) is then added at 25 μL/well and the OD's are read at 490 nm via an automated plate spectrophotometer.

Detection of Completely Human Immunoglobulins in Hybridoma Supernates

Growth positive hybridomas secreting fully human immunoglobulins can be detected using a suitable EIA. Briefly, 96 well pop-out plates (VWR, 610744) can be coated with 10 μg/mL goat anti-human IgG Fc in sodium carbonate buffer overnight at 4° C. The plates are washed and blocked with 1% BSA-PBS for one hour at 37° C. and used immediately or frozen at −20° C. Undiluted hybridoma supernatants are incubated on the plates for one hour at 37° C. The plates are washed and probed with HRP labeled goat anti-human kappa diluted 1:10,000 in 1% BSA-PBS for one hour at 37° C. The plates are then incubated with substrate solution as described above.

Determination of Fully Human Anti-IL-12 Reactivity

Hybridomas, as above, can be simultaneously assayed for inactivity to IL-12 using a suitable RIA or other assay. For example, supernatants am incubated on goat anti-human IgG Fc plates as above, washed and then probed with radiolabeled IL-12 with appropriate counts per well for 1 hour at RT. The wells are washed twice with FBS and bound radiolabeled IL-12 is quantitated using a suitable counter.

Human IgG1κ anti-IL-12 secreting hybridomas can be expanded in cell culture and serially subcloned by limiting dilution. The resulting clonal populations can be expanded and cryopreserved in freezing medium (95% FBS, 5% DMSO) and stored in liquid nitrogen.

Isotyping

Isotype determination of the antibodies can be accomplished using an EIA in a format similar to that used to screen the mouse immune sera for specific titers. IL-12 can be coated on 96-well plates as described above and purified antibody at 2 μg/mL can be incubated on the plate for one hour at RT. The plate is washed and probed with HRP labeled goat anti-human IgG_1 or HRP labeled goat anti-human IgG_3 diluted at 1:4000 in 1% BSA-PBS for one hour at RT. The plate is again washed and incubated with substrate solution as described above.

Binding Kinetics of Human Anti-Human IL-12 Antibodies with Human IL-12

Binding characteristics for antibodies can be suitably assessed using an IL-12 capture EIA and BIAcore technology, for example. Graded concentrations of purified human IL-12 antibodies can be assessed for binding to EIA plates coated with 2 μg/mL of IL-12 in assays as described above. The OD's can be then presented as semi-log plots showing relative binding efficiencies.

Quantitative binding constants can be obtained, e.g., as follows, or by any other known suitable method. A BIAcore CM-5 (carboxymethyl) chip is placed in a BIAcore 2000 unit. HBS buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% v/v P20 surfactant, pH 7.4) is flowed over a flow cell of the chip at 5 μL/minute until a stable baseline is obtained. A solution (100 μL) of 15 mg of EDC (N-ethyl-N'-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride) in 200 μL water is added to 100 μL of a solution of 2.3 mg of NHS (N-hydroxysuccinimide) in 200 μL water. Forty (40) μL of the resulting solution is injected onto the chip. Six μL of a solution of human IL-12 (15 μg/mL in 10 mM sodium acetate, pH 4.8) is injected onto the chip, resulting in an increase of ca. 500 RU. The buffer is changed to TBS/Ca/Mg/BSA running buffer (20 mM Tris, 0.15 M sodium chloride, 2 mM calcium chloride, 2 mM magnesium acetate, 0.5% Triton X-100, 25 μg/mL BSA, pH 7.4) and flowed over the chip overnight to equilibrate it and to hydrolyze or cap any unreacted succinimide esters.

Antibodies are dissolved in the running buffer at 33.33, 16.67, 8.33, and 4.17 nM. The flow rate is adjusted to 30 μL/min and the instrument temperature to 25° C. Two flow cells are used for the kinetic runs, one on which IL-12 had been immobilized (sample) and a second, underivatized flow cell (blank). 120 μL of each antibody concentration is injected over the flow cells at 30 μL/min (association phase) followed by an uninterrupted 360 seconds of buffer flow (dissociation phase). The surface of the chip is regenerated (interleukin-12/antibody complex dissociated) by two sequential injections of 30 μL each of 2 M guanidine thiocyanate.

Analysis of the data is done using BIA evaluation 3.0 or CLAMP 2.0, as known in the art. For each antibody concentration the blank sensogram is subtracted from the sample sensogram. A global fit is done for both dissociation (k_d, sec^{-1}) and association (k_a, mol^{-1} sec^{-1}) and the dissociation constant (K_D, mol) calculated (k_d/k_a). Where the antibody affinity is high enough that the RUs of antibody captured are >100, additional dilutions of the antibody are run.

Results and Discussion

Generation of Anti-Human IL-12 Monoclonal Antibodies

Several fusions are performed and each fusion is seeded in 15 plates (1440 wells/fusion) that yield several dozen antibodies specific for human IL-12. Of these, some are found to consist of a combination of human and mouse Ig chains. The remaining hybridomas secrete anti-IL-12 antibodies consisting solely of human heavy and light chains. Of the human hybridomas, all are expected to be Ig1κ.

Binding Kinetics of Human Anti-Human IL-12 Antibodies

ELISA analysis confirms that purified antibody from most or all of these hybridomas bind IL-12 in a concentration-dependent manner. FIGS. 1-2 show the results of the relative binding efficiency of these antibodies. In this case, the avidity of the antibody for its cognate antigen (epitope) is measured. It should be noted that binding IL-12 directly to the EIA plate can cause denaturation of the protein and the apparent binding affinities cannot be reflective of binding to undenatured protein. Fifty percent binding is found over a range of concentrations.

Quantitative binding constants are obtained using BIAcore analysis of the human antibodies and reveals that several of the human monoclonal antibodies are very high affinity with K_D in the range of 1×10^{-9} to 7×10^{-12}.

Conclusions

Several fusions are performed utilizing splenocytes from hybrid mice containing human variable and constant region antibody transgenes that are immunized with human IL-12. A set of several completely human IL-12 reactive IgG monoclonal antibodies of the IgG1κ isotype are generated. The completely human anti-IL-12 antibodies are further characterized. Several of generated antibodies have affinity constants between 1×10^{9} and 9×10^{12}. The unexpectedly high affinities of these fully human monoclonal antibodies make them suitable for therapeutic applications in IL-12-dependent diseases, pathologies or related conditions.

EXAMPLE 3

C340 is a Neutralizing Human Monoclonal Antibody

The bioactivity of IL-12 was shown to be neutralized by C340 in a variety of IL-12 dependent activity assays. Since IL-12 enhances IFN GAMMA production by NK cells and T lymphocytes, the effect of C340 antibody on the upregulation of IFN GAMMA mRNA and the effect of C340 on the production of IFN GAMMA protein was examined

(Trinchieri, G., Current Opinion in Immunology, 9:17–23 (1997), Morris, S. C., et al., Journal of Immunology, 152:1047–1056 (1994)). The ability of C340 to neutralize IL-12 driven induction of lymphokine activated killer (LAK) cell activity was also investigated in these studies (Kutza, J. and Murasko, D. M., Mechanisms of Ageing and Development, 90:209–222 (1996), Stem, A. S., et al., Proceedings of the National Academy of Sciences of the U.S.A., 87:6808–6812 (1990)). Lastly, the effect of C340 on IL-12-mediated upregulation of CD95 cell surface expression on T and NK cells was tested (Medvedev, A. E., et al., Cytokine, 9:394–404 (1997)).

Inhibition of IFN Gamma mRNA Transcription

To determine whether C340 inhibits IL-12/IL-2 induced IFN GAMMA gene transcription in human PBL, a reverse transcription-PCR assay was performed. Specific primers for β-actin (a control for mRNA integrity and content) and IFN GAMMA were used to amplify the cDNA obtained from stimulated human PBL. FIG. **3** shows C340 down regulates IFN GAMMA mRNA in IL-12/IL-2 activated (2 hour) PBMC.

Inhibition of Intracellular IFN GAMMA as Measured by Flow Cytometry

In response to various signals and as a measure of activation, T cells and NK cells can be induced to secrete cytokines. More specifically, PBL treated with IL-2 and IL-12 initiate substantial synthesis of IFN gamma within 4–8 hours after stimulation. This production can be detected in the cytoplasm of Brefeldin-A treated PBL by flow cytometry. FIG. **4** demonstrates a 60% reduction in IFN GAMMA production in such cultures when C340 IL-12 was added in conjunction with IL-12 for five hours.

Inhibition of IL-12 Induced IFN GAMMA Secretion

FIG. **5** clearly shows that two different lots of C340 inhibited the secretion of IFN GAMMA by peripheral blood lymphocytes in a dose-dependent fashion. Four hundred picograms of IL-12 were premixed with varying amounts of C340 and then added to IL-2 stimulated cultures of PBL's. When IFN GAMMA was measured by EIA after an 18–24 hour incubation, markedly diminished amounts of IFN GAMMA were detected with as little as 1 μg/mL of C340 antibody.

Inhibition of IL-12 Induced LAK Cell Cytotoxicity

Raji cells, an IL-12 sensitive Burkitt lymphoma derived cell line, is an NK cell resistant, LAK cell sensitive cell line. Raji cells, in triplicate, were cultured for four hours with LAK cells which had been activated with 400 pg/mL IL-12 and 10 U/mL IL-2 in the presence or absence of the human monoclonal antibody C340 (5000 ng/mL or 50 ng/mL). FIG. **6** shows the results from three normal, healthy donors. IL-12+IL-2 activation of effector cells resulted in an increasing cytotoxic activity over that of cells activated with IL-2 alone. The C340 antibody inhibited this IL-12 dependent effect. The magnitude of inhibition was related to antibody concentration, with the highest concentration tested reducing cytotoxicity to background levels.

Inhibition of CD95 Upregulation

Reports have described IL-12-induced upregulation of CD95 on the surface of highly purified CD56+ PBL. As can be seen in FIGS. **7A** and **7B**, distributional flow cytometric analysis revealed that CD95 expression was significantly upregulated on CD3+ T cells and CD56+ NK cells after treatment with IL-12 plus IL-2 for 72 hours. Concomitant anti-IL-12 treatment inhibited CD95 expression in both CD3+ and CD56+ populations. CD3+ cells were inhibited by ~50% (FIG. **7A**), whereas CD56+ cells were inhibited by ~85% (FIG. **7B**), as evidenced by a diminished MFI index (percent greater then unstimulated control).

EXAMPLE 4

Gene Cloning and Characterization

Genomic DNA fragments containing either the C340 heavy chain gene or the C340 light chain were cloned and purified. Genomic DNA purified from C340 hybridoma cells was partially digested with Sau3A restriction enzyme and size-selected by centrifugal fractionation through a 10–40% sucrose gradient. DNA fragments in the size range of 15–23 kb were cloned into the bacteriophage vector, EMBL3, and packaged into phage particles. Several packaging reactions resulted in a library of 1 million bacteriophage clones. Approximately 600,000 clones from the library were screened by plaque hybridization using 32P-labeled genomic DNA fragments that contained either human IgG 1 heavy chain constant region sequences or human kappa light chain constant region sequences as probe. Thirteen heavy chain and nine light chain clones were detected. Of these, three heavy chain clones and four light chain clones were purified by two more rounds of screening. One of the heavy chain clones and two of the light chain clones were shown to contain the 5' and 3' ends of the coding sequences by PCR analysis of bacteriophage DNA. The DNA insert in heavy chain (HC) clone H4 was 16 kb in size and includes 3.6 kb of 5' flanking and at least 2 kb of 3' flanking sequence. The DNA insert in light chain (LC) clone LC1 was 15 kb in size and included 4.4 kb of 5' flanking and 6.0 kb of 3' flanking sequence. The complete inserts were removed from the bacteriophage vector as SalI fragments and cloned between the XhoI and SalI sites of plasmid expression vector p1351, which provided a gpt selectable marker gene. Because there was an internal SalI site in the heavy chain variable region coding sequence, two SalI fragments had to be transferred from bacteriophage H4 to the p1351 expression vector. The resulting heavy and light chain expression plasmids were termed p1560 and p1558, respectively. The orientations of the heavy and light chain genes in these two plasmids relative to the p1351 vector sequences were determined using restriction enzyme analysis and PCR, respectively. In both cases, the orientations were such that the 5' end of the Ab gene fragment was proximal to the 3' end of the gpt gene. Both strands of the coding regions of the cloned genes were sequenced. The sequences of plasmids p1560 and p1558 are presented in FIGS. **11A–11K** and FIGS. **13A–13J**, respectively.

EXAMPLE 5

Preparation of Recombinant Cell Lines

Heavy chain plasmid p1560 was linearized by digestion with PvuI restriction enzyme and light chain plasmid p1558 was linearized using SalI restriction enzyme. p3X63Ag8.653 (653) and SP2/0-Ag14 (SP2/0) cells were separately transfected with the premixed linearized plasmids by electroporation and cells cultured and transfectants selected using mycophenolic acid as described (Knight, et al., Molecular Immunology 30:1443 (1993)). Cell supernatants from mycophenolic acid-resistant colonies were assayed approximately two weeks later for human IgG (i.e., recombinant C340 (rC340)). For this, cell supernatants were incubated on 96-well ELISA plates that were coated with goat antibodies specific for the Fc portion of human IgG. Human IgG which bound to the coated plate was detected using alkaline phosphatase-conjugated goat anti-human IgG

(heavy chain+light chain) antibody and alkaline phosphatase substrates as described (Knight, et al., Molecular Immunology 30:1443 (1993)). Cells of the higher producing clones were transferred to 24-well culture dishes in standard media and expanded (IMDM, 5% FBS, 2 mM glutamine, mycophenolic acid selection mix). The amount of antibody produced (i.e., secreated into the media of spent cultures) was carefully quantified by ELISA using purified C340 mAb as the standard. Selected clones were then expanded in T75 flasks and the production of human IgG by these clones was quantified by ELISA. Based on these values, six independent 653 transfectants and three independent SP2/0 transfectants were subcloned (by seeding an average of one cell per well in 96 well plates), the quantity of antibody produced by the subclones was determined by assaying (ELISA) supernatants from individual subclone colonies. Three subclones, 653 transfectant 19–20 (C379B) and the SP2/0 transfectants 84–81 (C381A) and 22–56 (C389A), were selected for further analysis.

Assay for rC340 Antigen Binding.

Prior to subcloning selected cell lines as described above, cell supernatants from three parental lines (653 transfectants clone 2 and clone 18 and SP2/0 transfectant clone 1) were used to test the antigen binding characteristics of rC340. The concentrations of rC340 in the three cell supernatant samples were first determined by ELISA. Titrating amounts of the supernatant samples, or purified C340 positive control, were then incubated in 96-well plates coated with 2 μg/ml of human IL-12. Bound mAb was then detected with alkaline phosphatase-conjugated goat anti-human IgG (heavy chain+light chain) antibody and the appropriate alkaline phosphatase substrates. As shown in FIG. 8, rC340 bound specifically to human IL-12 in a manner indistinguishable from the original C340 mAb.

Characterization of Selected Cell Lines.

Growth curve analyses were performed on C379B, C381A, and C389A by seeding T75 flasks with a starting cell density of 2×10^5 cells/ml in standard media or SFM-5 serum-free media and then monitoring cell number and rC340 concentration on a daily basis until the cultures were spent. The results of cultures in standard media are shown in FIGS. 9A-9C. Maximal C340 mAb production levels for C379B, C381A, and C389A were 135 μg/ml, 150 μg/ml, and 110 μg/ml, respectively. Attempts to adapt C379B cells to SFM-5 media were not successful. C381A cells produced the same amount of rC340 in SFM-5 media as in standard media, whereas C389A cells produced only half as much rC340 in SFM-5 media as in standard media.

The stability of rC340 mAb production over time for the three subclones was assessed by culturing cells in 24-well dishes with standard media or standard media without mycophenolic acid selection for varying periods of time. Lines C379B and C381A were observed to stably produce rC340 in the presence or absence of selection for a period of 30 days (the maximum time tested) and 75 days, respectively. Line C389A was unstable and after 43 days of culture produced just 20% as much antibody as at the beginning of the study.

It will be clear that the invention can be practiced otherwise than as particularly described in the foregoing description and examples.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

SEQUENCE LISTING

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Gln Gly Gln Val Thr Met Ser Val Asp Lys Ser Ile Thr Thr Ala Tyr
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Leu Gln Trp Asn Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
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            20                  25                  30

Ala Arg Gln Thr Leu Glu Phe Tyr Pro Cys Thr Ser Glu Glu Ile Asp
        35                  40                  45

His Glu Asp Ile Thr Lys Asp Lys Thr Ser Thr Val Glu Ala Cys Leu
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Pro Leu Glu Leu Thr Lys Asn Glu Ser Cys Leu Asn Ser Arg Glu Thr
65                  70                  75                  80

Ser Phe Ile Thr Asn Gly Ser Cys Leu Ala Ser Arg Lys Thr Ser Phe
                85                  90                  95

Met Met Ala Leu Cys Leu Ser Ser Ile Tyr Glu Asp Leu Lys Met Tyr
            100                 105                 110

Gln Val Glu Phe Lys Thr Met Asn Ala Lys Leu Leu Met Asp Pro Lys
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Leu His Ala Phe Arg Ile Arg Ala Val Thr Ile Asp Arg Val Met Ser
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        195                 200                 205

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225                 230                 235                 240

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What is claimed is:

1. An isolated anti-IL-12 antibody, comprising a heavy chain variable region (V_H) of the amino acid sequence set forth in SEQ ID NO:7 and a light chain variable region (V_L) of the amino acid sequence set forth in SEQ ID NO:8.

2. The anti-IL-12 antibody according to claim 1, wherein said antibody binds IL-12 with an affinity of at least one selected from at least 10^{-9} M, at least 10^{-10} M, at least 10^{-11} M, or at least 10^{-12} M.

3. The anti-IL-12 antibody according to claim 1, wherein said antibody neutralizes an activity of IL-12 protein.

4. A composition comprising an isolated anti-IL-12 antibody having a heavy chain variable region (V_H) of the amino acid sequence set forth in SEQ ID NO:7 and a light chain variable region (V_L) of the amino acid sequence set forth in SEQ ID NO:8, and at least one pharmaceutically acceptable carrier or diluent.

* * * * *

Exhibit 3

Copy of U.S. Patent & Trademark Office Maintenance Fee Statements for U.S. Patent No. 6,902,734

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ONE JOHNSON & JOHNSON PLAZA
NEW BRUNSWICK NJ 08933-7003

MAINTENANCE FEE STATEMENT

According to the records of the U.S.Patent and Trademark Office (USPTO), the maintenance fee and any necessary surcharge have been timely paid for the patent listed below. The "PYMT DATE" column indicates the payment date (i.e., the date the payment was filed).

The payment shown below is subject to actual collection. If the payment is refused or charged back by a financial institution, the payment will be void and the maintenance fee and any necessary surcharge unpaid.

Direct any questions about this statement to: Mail Stop M Correspondence, Director of the USPTO, P.O.Box 1450, Alexandria, VA 22313-1450.

PATENT NUMBER	FEE AMT	SUR CHARGE	PYMT DATE	U.S. APPLICATION NUMBER	PATENT ISSUE DATE	APPL. FILING DATE	PAYMENT YEAR	SMALL ENTITY?	ATTY DKT NUMBER
6,902,734	**$980.00**	**$0.00**	**11/06/08**	**09/920,262**	**06/07/05**	**08/01/01**	**04**	**NO**	**CENTOCOR, INC. - USA I186**

Exhibit 4 - Claims 1-4 of U.S. Patent No. 6,902,734 Read on the Active Ingredient and a Composition of the Approved Product.

Claims	STELARA™ Properties and Description
What is claimed is: 1. An isolated anti-IL-12 antibody, comprising a heavy chain variable region (V_H) of the amino acid sequence set forth in SEQ ID NO:7 and a light chain variable region (V_L) of the amino acid sequence set forth in SEQ ID NO:8.	Ustekinumab (the active ingredient of STELARA™) is an isolated anti-IL-12 antibody. Ustekinumab comprises a heavy chain variable region amino acid sequence corresponding to SEQ ID NO:7 of the '734 Patent and a light chain variable region amino acid sequence corresponding to SEQ ID NO:8 of the '734 Patent.
2. The anti-IL-12 antibody according to claim 1, wherein said antibody binds IL-12 with an affinity of at least one selected from at least 10^{-9} M, at least 10^{-10} M, at least 10^{-11} M, or at least 10^{-12} M.	Ustekinumab binds IL-12 with an affinity of at least 10^{-9} M, at least 10^{-10} M, at least 10^{-11} M, or at least 10^{-12} M.
3. The anti-IL-12 antibody according to claim 1, wherein said antibody neutralizes an activity of IL-12 protein.	Ustekinumab has been shown to neutralize an activity of the IL-12 protein, including, without limitation neutralizing IL-12 induced IFNγ production.
4. A composition comprising an isolated anti-IL-12 antibody having a heavy chain variable region (V_H) of the amino acid sequence set forth in SEQ ID NO:7 and a light chain variable region (V_L) of the amino acid sequence set forth in SEQ ID NO:8, and at least one pharmaceutically acceptable carrier or diluent.	The STELARA™ composition comprises Ustekinumab (an isolated anti-IL-12 antibody comprising a heavy chain variable region amino acid sequence corresponding to SEQ ID NO:7 and a light chain variable region amino acid sequence corresponding to SEQ ID NO:8) formulated as follows with at least one pharmaceutically acceptable carrier or diluent: STELARA™ contains either 45 mg (0.5 mL vial) or 90 mg (1 mL vial) of ustekinumab for subcutaneous use formulated with sucrose, L-histidine, and polysorbate 80.

Exhibit 5

DESCRIPTION OF SIGNIFICANT ACTIVITIES OF APPLICANT DURING REGULATORY REVIEW

Summary of Significant Events During Regulatory Review Period

Centocor and the FDA have collaborated extensively during the development of STELARA™ (ustekinumab or CNTO 1275) in the treatment of plaque psoriasis. The table below contains a high level summary of significant FDA interactions and agreements

Summary of Significant Events

Date	Event
2 November 2000	Pre-IND meeting
09 November 2000	Submission of meeting minutes from the Pre-IND Telephone contact of 02 November 2000
15 December 2000	Authorization letter for the FDA to cross-reference another company's Drug Master File for information about a compound in connection with the IND for 12b75.
27 December 2000	Submission of Initial Investigational New Drug Application
08 January 2001	FDA Letter assigning BB-IND 9590 for Psoriasis
26 January 2001	Teleconference between Centocor and FDA regarding rules for overlapping treatment and location and investigator for proposed study
15 February 2001	FDA letter stating the clinical study may proceed, and a request for further Chemistry, Manufacturing and Controls information
15 March 2001	Information requested from Cilag AG regarding shipment of final vialed product
20 March 2001	On hold shipment release notification
28 March 2001	Protocol Amendment, Change in Protocol: Amendment 1 to protocol submitted along with new investigator, Certificate of Analysis for clinical trial lot and revised infusion bag label
19 April 2001	Response to FDA request for information: Response to FDA request dated 15 February
25 July 2001	Protocol Amendment, Change in Protocol: Amendment 2 to protocol
12 October 2001	Fax from Centocor to FDA providing summary of preliminary safety and PK data from the Phase 1 study

Summary of Significant Events

Date	Event
12 October 2001	Fax from Centocor to FDA providing a status report on the on-going developmental toxicology study and a copy of the study protocol
18 October 2001	FDA email request for information regarding study enrollment
19 October 2001	Centocor email response to request for regarding study enrollment
26 October 2001	Fax from Centocor to FDA update on the developmental toxicology study
03 December 2001	Information Amendment: Chemistry, Manufacturing and Controls-stability update
20 Feb 2002	Information Amendment: Chemistry Manufacturing and controls information regarding the new lyophilized formulation
26 February 2002	Information Amendment: Clinical - Updated clinical investigator's brochure submitted
22 March 2002	Protocol Amendment: Change in Protocol - Amendment 3 to ongoing study protocol and New Protocol for next study Corrected Cross reference DMF IND Annual Report covering 27 January 2001-26 January 2002
18 April 2002	Information Amendment: Clinical - Revised model informed consent form for second study
15 May 2002	Information Amendment: Chemistry / Microbiology - certificate of analysis for CNTO 1275 to be used in second study
19 June 2002	Information Amendment: Chemistry / Microbiology Updated Stability Data
05 September 2002	Protocol Amendment: Change in Protocol - Amendment 1 to Protocol for second study
04 Feb 2003	Centocor to FDA email providing a status report on the embryo fetal development toxicity study in monkeys
12 March 2003	Annual Report covering 27 January 2002 through 26 January 2003
01 April 2003	Information Amendment: Clinical - Updated clinical investigators brochure (CIB)
28 April 2003	Protocol Amendment: New Protocol, Model Informed Consent Form, Vial And Carton Labels

Summary of Significant Events

Date	Event
28 April 2003	Information Amendment: Chemistry / Microbiology - Revised Chemistry, Manufacturing And Controls information for CNTO 1275 drug substance and drug product and placebo for ongoing study
09 May 2003	Centocor fax to FDA following up on a previous telephone discussion regarding cell line switch
12 May 2003	Centocor email regarding FDA review of cell line switch proposal
13 May 2003	Centocor email to FDA: contact report regarding comments on the protocol and informed consent form for ongoing study
19 May 2003	Information Amendment: Clinical - Revised informed consent form for ongoing study
28 May 2003	Email and telephone discussion regarding CNTO 1275 cell line switch proposal.
30 May 2003	Information Amendment: Pharmacology/Toxicology: Final Study Report
02 June 2003	Information Amendment: Chemistry / Microbiology and Clinical - Certificates of analysis for clinical lots and syringe labels for ongoing Phase 2 study
18 June 2003	Email and telephone discussions with the FDA CMC reviewer
26 June 2003	Centocor email to FDA with additional information regarding pharmacokinetic (PK) study to support the cell line switch.
18 July 2003	FDA email response regarding additional information for CNTO 1275 pharmacokinetic (PK) study to support the cell line switch.
12 August 2003	Information amendment: Pharmacology / Toxicology - final study report submission
05 October 2003	FDA mail follow up response regarding toxicology study
16 October 2003	Correspondence: Response to the 08-Oct-2003 FDA request for additional data supporting references in toxicology and historical background data
16 October 2003	Centocor to FDA email summary of information previously submitted regarding toxicology study
23 October 2003	Information Amendment: Clinical - Revised model informed consent form for ongoing study

Summary of Significant Events

Date	Event
13 November 2003	Centocor email to FDA- regarding additional toxicity studies in 2004
18 December 2003	Centocor fax to FDA regarding the Investigator's Brochure
05 January 2004	Centocor email to FDA regarding toxicology study
04 February 2004	Email - FDA request for half-life information and response
19 March 2004	Annual Report covering 27-Jan-2003 through 26-Jan-2004
23 March 2004	Information Amendment: Clinical - Updated Clinical Investigators Brochure (CIB) For CNTO 1275 Dated 19-Mar-2004
25 March 2004	Information Amendment: Chemistry / Microbiology - characterization data comparing the new cell line with the original cell line
30 March 2004	Information Amendment: Clinical - final Clinical Study Report
07 April 2004	Information Amendment: Clinical - Updated Clinical Investigator's Brochure (CIB) dated 02 April 2004
07 May 2004	Information Amendment: Chemistry/Microbiology - Information and manufacturing changes submitted in the common technical document format (CTD)
17 November 2004	Information Amendment: Clinical - preliminary results from ongoing psoriasis study
25 March 2005	Meeting request for End of Phase 2 meeting was submitted.
25 March 2005	Annual Report covering 27 January 2004 through 26 January 2005
25 April 2005	Correspondence: The End of Phase 2 meeting briefing document.
26 May 2005	End of Phase 2 meeting - The FDA provided Centocor with significant feedback and guidance on the clinical development plan.
30 September 2005	Protocol Amendment: New Protocol: Phase 3 Protocol submitted
25 October 2005	Type C Meeting regarding plans for new manufacturing facility

Summary of Significant Events

Date	Event
15 November 2005	Information Amendment: Chemistry/Microbiology: Centocor has developed a liquid formulation. This amendment notifies FDA of the following changes: • Introduction of new drug substance manufacturing facility. • Updated methods, in-process and release specifications, analytical methods, stability data, and adventitious agents safety evaluation. • Comparability data.
23 November 2005	Information Amendment: Chemistry/Microbiology Centocor has developed a liquid formulation. This amendment notifies FDA of the following changes: • Introduction of new drug product manufacturing facility. • Change to a liquid formulation • Updated container closure system, analytical methods, specifications • Change in placebo formulation
08 December 2005	Protocol Amendment: New Protocol - Phase 3 Protocol submitted
21 December 2005	Correspondence: Follow-up to End of Phase 2 request
20 March 2006	Protocol Amendment: New Protocol was submitted
07 and 14 June 2006	Informal FDA Teleconference (Product Reviewer): Centocor discussed its proposed stability strategy
22 August 2006	Correspondence Type A meeting request and briefing document
06 September 2006	FDA written correspondence: Type A meeting date was set.
13 October 2006	Type C meeting: discussion of potential regulatory pathways.
09 November 2006	Correspondence: a pre-BLA meeting request was submitted with draft questions. The meeting was subsequently scheduled for 14 March 2007.
21 November 2006	Informal FDA contact: FDA called to inform Centocor that the Type A meeting scheduled for 27 November was being canceled and was to be rescheduled.

Summary of Significant Events

Date	Event
27 November 2006	Type A meeting: FDA canceled the type A meeting and would follow-up to reschedule.
06 December 2006	Informal teleconference with FDA: Medical reviewer requested an overview of the clinical development program including overview of the clinical plan, summary of safety, summary of the Phase 3 studies.
01 February 2007	Information Amendment: Chemistry/Microbiology • Introduction of Baxter Pharmaceutical Solutions LLC as a Pre-filled syringe production site. • Update to manufacturing methods, analytical methods, specifications and container closure, and stability data for a pre-filled syringe.
8 February 2007	FDA FAX correspondence: the FDA provided written comments on the 22 August briefing document in lieu of granting a formal meeting.
23 February 2007	Information Amendment: Pharmacology/Toxicology - Preclinical proposed study designs submitted for review
14 March 2007	Pre-BLA Meeting FDA provided detailed guidance and feedback on the content and format of Centocor's planned BLA for CNTO 1275 in the treatment of psoriasis. Agreements were reached on safety and efficacy analyses to be included in the submission.
14 May 2007	Pre-BLA Meeting (CMC):
23 May 2007	Correspondence: Centocor submitted a proposed proprietary name for premarketing review and assessment.
24 September 2007	Type A Meeting (Follow-up to Pre-BLA Meeting)
26 September 2007	Type A Meeting (follow-up to Pre-BLA Meeting):
28 November 2007	BLA Filing of BLA 125261
17 June 2008	Advisory Committee Meeting
29 July 2008	FDA extended the goal date by 3 months to 28 Dec 2008
18 December 2008	Complete Response letter received; No new clinical data was requested.
09 January 2009	Centocor submitted the Response to the FDA CR letter

Summary of Significant Events

Date	Event
11 March 2009	Meeting with CMC for clarification and feedback
23 March 2009	Centocor Response to information request dated 18 March 2009
27 March 2009	Centocor CMC response to the FDA information request dated 23 Feb 2009
19 May 2009	FDA extension letter - user fee goal date has been extended 3 months to 09-Oct-2009
26 June 2009	Centocor submitted Proposed REMS (Risk Evaluation and Mitigation Strategy) and REMS supporting document incorporating FDA feedback
28 July 2009	FDA provided Postmarketing Requirements and Commitments
05 August 2009	Centocor submitted response to FDA proposed Postmarketing Requirements and Commitments
08 September 2009	FDA provided draft Medication Guide comments
22 September 2009	FDA provided final draft REMS
25 September 2009	FDA provided the approval letter for the Original BLA for STELARA™

Exhibit 6

Power of Attorney Appointing Eric A. Dichter
As Representative of the Owner of the '734 Patent

1F12 1646

DOCKET NO. CEN0248

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	David M. Knight, et al.		
Serial No.:	09/920,262	Art Unit:	1646
Filed:	August 1, 2001	Examiner:	P. M. Mertz
For:	ANTI-IL-12 Antibodies, Compositions, Methods and Uses		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ASSOCIATE POWER OF ATTORNEY

Dear Sir:

In the matter of the above-identified application, I hereby appoint Eric Dichter (Reg. No. 41,708), whose postal address is One Johnson & Johnson Plaza, New Brunswick, New Jersey 08933-7003, my associate attorney to prosecute said application, to make alterations and amendments therein, to file continuing applications claiming the benefit of said application, to receive the patent and to transact all business in the Patent Office connected with said application.

I request all communications with respect to said application be addressed to Philip S. Johnson, One Johnson & Johnson Plaza, New Brunswick, New Jersey 08933-7003. All future telephone calls should be directed to Eric Dichter at 610.651.7491.

Signed at New Brunswick, in the County of Middlesex and State of New Jersey, this 4th day of June, 2004.

Guy Kevin Townsend
Registration No. 34,033
Attorney for Applicant(s)

Johnson & Johnson
One Johnson & Johnson Plaza
New Brunswick, NJ 08933-7003
732.524.2517
Dated: June 4, 2004

Please type a plus sign (+) inside this box [+]

PTO/SB/01 (10-00)
Approved for use through 10/31/2002. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

SUBSTITUTE DECLARATION AND POWER OF ATTORNEY FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☐ Declaration Submitted with Initial Filing **OR** ☒ Declaration Submitted after Initial Filing (Surcharge (37 CFR 1.16(e)) required)

Attorney Docket Number	CEN0248
First Named Inventor	David M. Knight, et al.
COMPLETE IF KNOWN	
Application Number	09/920,262
Filing Date	August 1, 2001
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Anti-Il-12 Antibodies, Compositions, Methods And Uses
(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 8/1/2001 as United States Application Number or PCT International Application Number 09/920,262.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	Certified Copy Attached? NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

DECLARATION - Utility or Design Patent Application

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	
60/223,358 60/236,827	08/07/2000 09/29/2000	☐ Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status
		Patented Patented Patented

I hereby appoint:

☒ Practitioners at Customer Number **000027777** →

Place Customer Number Bar Code Label Here

AND

☐ Practitioner(s) named below:

Name — Registration Number

as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

Address all telephone calls to Guy Kevin Townsend at telephone number (732) 524-2517.

Direct all correspondence to: ☒ Customer Number or Bar Code Label **000027777** OR ☐ Correspondence address below

Name:		
Address:		
Address:		
City:	**State:**	**ZIP**
Country	**Telephone:**	**Fax:**

Exhibit 7

STATEMENT THAT APPLICANT IS ELIGIBLE
FOR EXTENSION AND LENGTH OF EXTENSION CLAIMED

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No.: 6,902,734 Issued: June 7, 2005
Expiration Date: July 27, 2022

Inventors: Jill Giles-Komar; David M. Knight; David Peritt; Bernard Scallon; David Shealy

Title: ANTI-IL-12 ANTIBODIES AND COMPOSITIONS THEREOF

Statement of Eligibility for Extension of
Patent Term Due to Regulatory Review

I, Eric A. Dichter, represent that I am the attorney of record duly appointed by the assignee of the entire right, title and interest in the patent application identified above, and do state on behalf of the Applicant as follows:

To the best of my knowledge, U.S. Patent No. 6,902,734 (the '734 Patent) meets all of the eligibility criteria set forth in 37 C.F.R §§ 1.710 and 1.720 for extension of patent term.

The '734 Patent claims a "product" as that term is defined in 37 C.F.R § 1.710, specifically the active ingredient, ustekinumab, and the composition of a new human drug, STELARA™ (ustekinumab) 37 C.F.R § 1.720(a).

The term of the '734 Patent has never been previously extended. 37 C.F.R § 1.720(b).

An application for extension of the term of the '734 Patent in compliance with 37 C.F.R § 1.740 is herewith submitted. 37 C.F.R § 1.720(c).

The approved product, STELARA™ (ustekinumab), has been subject to a regulatory review period before its commercial marketing or use as defined in 35 U.S.C. §156(g). 37 C.F.R § 1.720(d).

The approved product, STELARA™ (ustekinumab), has received permission for commercial marketing or use and the permission for the commercial marketing or use of the product is the first received permission for commercial marketing or use under the provision of law under which the applicable regulatory review occurred. 37 C.F.R §1.720(e).

The application for extension of the term of the '734 Patent submitted herewith is submitted within the sixty-day period beginning on the date the product first received permission for commercial marketing or use under the provisions of law under which the applicable regulatory review period occurred. 37 C.F.R §1.720(f).

The term of the '734 Patent, including any interim extension issued pursuant to § 1.790, has not expired before the submission of an application in compliance with 37 C.F.R. § 1.741. 37 C.F.R §1.720(g).

No other patent term has been extended for the same regulatory review period for the approved product, STELARA™ (ustekinumab), 37 C.F.R §1.720(h).

Extension Calculation

The extension claimed is 425 days, setting the patent to expire on September 25, 2023. The following are the calculations, made in accordance with 37 C.F.R. § 1.775, that result in the claimed extension:

(1) The testing phase began on December 28, 2000 (the effective date of the IND) and ended on November 28, 2007 (submission date of the BLA) = 2526 days.

(2) The approval phase began on November 29, 2007 (date of receipt by the FDA of the BLA) and ended on September 25, 2009 (the date the approval was granted); the total number of days in the approval phase (from and including November 29, 2007 to and including September 25, 2009) is 667 days from the start date to the end date, end date included.

(3) The regulatory review period is the sum of the testing phase and approval phase (2526 + 667) = 3193 days.

(4) The '734 Patent issued on June 7, 2005 during the regulatory period (1622 days after the effective date of the IND).

(5) Applicant acted with due diligence throughout the entire regulatory review period.

(6) The term that the patent is extended is determined by subtracting from the regulatory review period (3193 days):

(i) the number of days in the regulatory review period on or before the date on which the patent issued (1622 days),

(ii) the number of days during which it is determined that Applicant did not act with due diligence (0 days), and

(iii) ½ the number of days remaining in the testing phase after it is reduced by the number of days in the regulatory review period on or before the date on which the patent issued (½ of 904 = 452 days) = (3193 - 1622 - 0 - 452 = 1119) 1119 days.

(7) The original expiration date of the patent is July 27, 2022.

(8) Addition of the extension of 1119 days to the original expiration date of the patent would extend the expiration date of the patent to August 19, 2025.

(9) Fourteen years from the approval date of the product (September 25, 2009) is September 25, 2023.

(10) Pursuant to 35 U.S.C. § 156(c)(3), the extended term of the patent cannot exceed 14 years from the date of product approval. The fourteen year cap applies here since the extension of 1119 days sets the patent to expire (August 19, 2025) after the date that is 14 years post-approval (September 25, 2023).

(11) Pursuant to 35 U.S.C. § 156(g)(6)(A), the extension period is subject to a five year limitation (for patents issued after September 24, 1984). The five year limitation does not apply since the extension of 425 days is less than five years.

(12) The '734 Patent is entitled to an extension of 425 days.

(13) In light of the conclusions set forth above, the extended expiration date of the '734 Patent is believed to be September 25, 2023.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 20 November 2009

/Eric Dichter/

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